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ACUTE POSTOPERATIVE ESOPHAGEAL, GASTRIC AND DUODENAL ULCERATIONS

A FURTHER STUDY OF THE PATHOLOGIC CHANGES IN SHOCK

ABRAHAM PENNER, M.D.

AND

ALICE IDA BERNHEIM, M.D.

NEW YORK

In a previous study in which we investigated the pathogenesis of acute postoperative enterocolitis we arrived at the conclusion that the mechanisms involved in the production of that condition were those which formed the bodily responses to shock.[1] In several instances we were impressed with the occurrence of acute duodenal, gastric and esophageal erosions or ulcers simultaneously with typical enterocolitis. We therefore decided to study a group of cases in which these esophageal, gastric and duodenal lesions were present, in order to determine whether their occurrence in association with enterocolitis was merely a coincidence or dependent on the same vasomotor mechanisms for their causation.

HISTORICAL REVIEW

The earliest report of a case of postoperative duodenal ulceration was communicated by Billroth[2] in 1867. He presented the case of a 42 year old man with an enormous substernal goiter which almost caused suffocation. The man's general condition was so poor that Billroth hesitated to operate and hence attempted to evacuate the fluid contents of the cystic gland. Since this was not successful, he operated and exposed the gland by incising the fascia over it so that the gland protruded. Owing to the condition of the patient and the fact that the gland extended substernally, nothing further was done. Twenty-four hours later there was abdominal pain, diarrhea, profuse perspiration and restlessness. Two days later the patient was in collapse, and on the fourth day it was noted that his bowel movements were tarry. He died

From the Laboratories of the Mount Sinai Hospital.

1. Penner, A., and Bernheim, A.: Arch. Path. **27**:966, 1939.
2. Billroth, C. A. T.: Wien. med. Wchnschr. **17**:705, 1867.

two days after this, with a terminal rise in temperature. At autopsy there were observed four acute ulcers in the duodenum. In his report Billroth mentioned that he had been told of 2 other cases; one of these was identical with his own; the other was a case of inguinal hernia which had become strangulated and was operated on after fourteen hours of vain attempts at reduction. At autopsy in the latter case several hemorrhagic infarcts were encountered in the stomach and two gastric ulcers symmetrically arranged with relation to the lesser curvature. Despite the fact that there were no clinical signs of sepsis and that in his own case he specifically noted that the wound was clean, Billroth considered that the lesions were embolic in origin and probably septic.

Twenty-two years later von Eiselsberg [3] reported a series of 8 cases of gastroduodenal bleeding occurring after operation. Of the patients, 3 survived. These patients had enormous hernias, which were finally reduced operatively. The fourth case was one of carcinoma of the rectum, which was resected. The patient went into collapse forty-eight hours after operation and died three days later. Autopsy showed diffuse peritonitis; there were also about 30 fresh hemorrhagic erosions in the stomach, of which 2 were about the size of a dime. The ulcers showed "nothing specific." His other cases were similar in nature and concerned incarcerated hernias. He considered the lesions to be the result of retrograde emboli from thromboses in the omentum. His interest in this subject persisted and was evidenced by the papers of Busse [4] and von Winiwarter [5] from his clinic. These papers were concerned mainly with the clinical features of postoperative intestinal hemorrhage. Their main importance lies in the fact that they record observations on cases in which the intestinal tract was not operated on. Thus von Winiwarter noted these lesions occurring after splenectomy and after operations for ectopic pregnancy, hypernephroma and retroperitoneal sarcoma. Despite this, they affirmed the etiologic significance of retrograde embolism.

Dieulafoy, who is frequently credited with the first description of postappendectomy gastroduodenal ulceration,[6a] noted the clinical occurrence of hematemesis after appendectomy. Although he made no postmortem observations in these cases, he considered the process identical with that observed in a case of gastric ulceration after strangulated hernia, in which he studied the gross and microscopic lesions. He grouped these with instances of "toxic-infectious" gastric ulcerations in which he had found pneumococci [6b] present in the lesions and with

3. von Eiselsberg, F.: Arch. f. klin. Chir. **59**:837, 1899.

4. Busse, W.: Arch. f. klin. Chir. **76**:122, 1905.

5. von Winiwarter, J. R.: Arch. f. klin. Chir. **95**:161, 1911.

6. Dieulafoy, G.: (*a*) Presse méd. **9**:73, 1901; (*b*) Gastrite ulcereuse pneumococcique, in Clinique médicale de l'Hôtel-Dieu de Paris, Paris, Masson & Cie, 1899, vol. 3; (*c*) Exulceratio simplex, ibid., vol. 2, p. 1.

his "exulceratio simplex." [6c] His clinical descriptions leave little doubt as to the presence of shock in his cases.

In 1902 Nietzsche [7] reported an interesting case of gastric hemorrhage occurring after appendectomy. The patient had been ill with peritoneal symptoms for three days before coming under observation. He was delirious, presented marked distention and regurgitation of coffee ground material, and had a weak pulse. He was not operated on. At autopsy generalized peritonitis was found. No mesenteric thromboses were observed. The stomach showed countless flat ulcers from pinhead to pea size distributed in the fundus and along the greater curvature and covered with clotted blood. Microscopically the mucosa appeared well preserved in the areas which were not grossly ulcerated. Throughout the stomach the vessels and capillaries of the submucosa were focally distended with blood. Occasionally, normal areas showed thrombosed venules in the submucosa. In addition, he observed focal accumulations of leukocytes—usually noted in otherwise normal areas of the mucosa, less often in the submucosa. Furthermore, he noted focal areas in which the free ends of the tubules showed necrosis. These focal necroses were interspersed between well preserved ones. He considered the lesions to be due to sepsis.

Similar lesions have been observed in the esophagus (Kaufmann,[8] 1909; Pringle, Stewart and Teacher,[9] 1921). In the series reported by Pringle, Stewart and Teacher the esophageal lesions occurred following postappendical peritonitis six times, three times subsequent to cholecystectomy for cholecystitis and pancreatitis and once as a result of a traumatic rupture of the liver with intra-abdominal hemorrhage. One instance was noted in which the lesion was found at autopsy following death from peritonitis due to perforation of a duodenal ulcer. In the opinion of these authors the lesions were the result of antemortem digestion of the esophagus following regurgitation of gastric contents. They expressed great surprise at the observation in their case of traumatic rupture of the liver with marked engorgement of the mucosal vessels and blood pigmentation of the tissues but no cellular reaction, findings which they attributed to the rapid collapse and death of the patient. Their protocols leave no doubt of the presence of shock in their cases.

More recently Bartels [10] was able to find 82 such cases in his study of a series of 6,000 esophageal specimens. Of these, 55 were postoperative. He noted that in these cases "it might be said that all the

7. Nietzsche, E.: Deutsche Ztschr. f. Chir. **64**:180, 1902.

8. Kaufmann, E.: Lehrbuch der speciellen pathologischen Anatomie, ed. 5, Berlin, G. Reimer, 1909, p. 388.

9. Pringle, J. H.; Stewart, L. T., and Teacher, J. H.: J. Path. & Bact. **24**: 396, 1921.

10. Bartels, E. C.: Arch. Path. **20**:369, 1935.

operations were rather difficult and extensive, and occasionally, the patients constituted poor surgical risks." Of the remaining 27 cases there were 13 of sepsis in which embolic phenomena could not be ruled out. In addition, there were 6 instances of chronic ulcerative colitis with severe debilitation, and, most interestingly, 1 instance of acute coronary thrombosis in which death occurred in twenty-four hours. He notes that "it seems necessary that the patient should be debilitated, but not necessarily dying before changes can occur in the esophagus." He attributes the lesions to the action of regurgitated gastric juice.

It is well known that about 45 per cent of instances of melena neonatorum vera are due to ulceration in the esophagus, stomach or duodenum (Shukowsky [11]). The pathogenesis of such ulcerations is obscure, but their presence in infants on the first day of life (Zadek [12]) makes a peptic factor unlikely. Individual case reports with complete autopsy protocols are not numerous. However, Pomorski [13] in 1892 reported the case of a premature child, born after a normal labor and apparently normal, who began to vomit blood two days after birth, went into collapse and died. Necropsy showed a large hemorrhage in the fourth ventricle with compression of the cerebellum. He also noted the remarkable hyperemia of the gastric and duodenal mucosa and the associated marked edema and focal hemorrhages. Microscopic study showed the marked edema of the mucosa and submucosa with hyperemia and punctiform mucosal hemorrhages. In addition, there was an ulcer in the cardiac end of the stomach which extended into the submucosa, as well as several punctiform hemorrhages in the small intestine. Similar conditions have been described by Vassmer,[14] Zadek,[12] Spiegelberg [15] and von Preuschen [16] as resulting from intracranial birth trauma.

The occurrence of such acute erosions and ulcerations in the stomach and duodenum has been repeatedly noted in consequence of severe cutaneous burns (Curling [17]). Harkins [18] presented a historical review of this subject and referred to a report by Swan in 1823 as the earliest record of such a case. Furthermore, similar lesions have been noted as occurring in instances of prolonged exposure to cold and resultant freezing (Adams [19]; Förster [20]). Despite the common opinion that these

11. Shukowsky, W. P.: Arch. f. Kinderh. **45**:321, 1907.
12. Zadek, I.: Arch. f. Verdauungskr. **18**:785, 1912.
13. Pomorski, J.: Arch. f. Kinderh. **14**:165, 1892.
14. Vassmer: Arch. f. Gynäk. **89**:275, 1909.
15. Spiegelberg, cited by Landau, L.: Ueber Melaena der Neugeborenen, nebst Bemerkungen über die Obliteration der foetalen Wege, Breslau, Maruschke und Berendt, 1874.
16. von Preuschen, F.: Centralbl. f. Gynäk. **18**:201, 1894.
17. Curling, T. B.: Med.-Chir. Tr. London **25**:260, 1842.
18. Harkins, H. N.: Surgery **3**:608, 1938.
19. Adams, S.: Am. M. Times **6**:101, 1863.
20. Förster, A.: Mitt. a. d. path. anat. Anstalt Würzburg **2**:155, 1861.

lesions are the result of post-traumatic sepsis it should be pointed out that the work of Underhill and his co-workers [21] as well as that of Davidson and Matthew [21a] indicates that the vascular changes as well as the alterations in the concentration of the various components of the blood are identical with those which occur in shock produced by other means. Similarly Harkins [18] has shown that blood concentration begins almost immediately after freezing has occurred, with a rise in hemoglobin values to 168 per cent, and only later leads to the fatal drop in blood pressure. Furthermore, in the cases reported by Adams [19] and Förster [20] there were no evidences of sepsis either clinically or at autopsy.

The acute ulcers and erosions of the esophagus, stomach and duodenum described by Cushing [22] and related by him to lesions in the interbrain or its various pathways appear to us to belong in this category. In analyzing his 11 case reports we excluded from our present consideration 2 cases (6 and 7) in which diffuse vascular disease was present (malignant hypertension) and 2 cases (10 and 11) in which chronic ulcers were observed. Analysis of the remaining 7 cases shows that the lesions followed intracranial operations. Most of these operations were for tumors located in the cerebellum, but one was for a tumor in the olfactory groove and one for an aneurysm of the basilar artery. Similarly the tumors varied in type; among them were included astrocytoma, medulloblastoma, angioblastoma and meningioma. In contrast to these variable factors we note the almost invariable large loss of blood in intracranial operations, which may amount to 500 to 900 cc. in a simple craniotomy and exploration and to over 2,000 cc. in removal of a large meningioma (White and co-workers [23]). Another accompaniment of craniotomy is the drop of intracranial pressure which occurs when the dura is incised. This leads to overactivity of the carotid sinus, resulting in vasoconstriction in the viscera and increase in the secretion of epinephrine (Ask-Upmark [24]). These are all components of the picture of shock.

REPORT OF CASES

In a survey of the postmortem records of the Mount Sinai Hospital for a period of approximately ten years we found 47 cases in which

21. Underhill, F. P.; Carrington, G. L.; Kapsinow, R., and Park, G. T.: Arch. Int. Med. **32**:31, 1923.

21a. Davidson, E. C., and Matthew, C. W.: Arch. Surg. **15**:265, 1927.

22. Cushing, H.: Peptic Ulcer and the Interbrain, in Papers Relating to the Pituitary Body, Hypothalamus and Parasympathetic Nervous System, Springfield, Ill., Charles C. Thomas, Publisher, 1932.

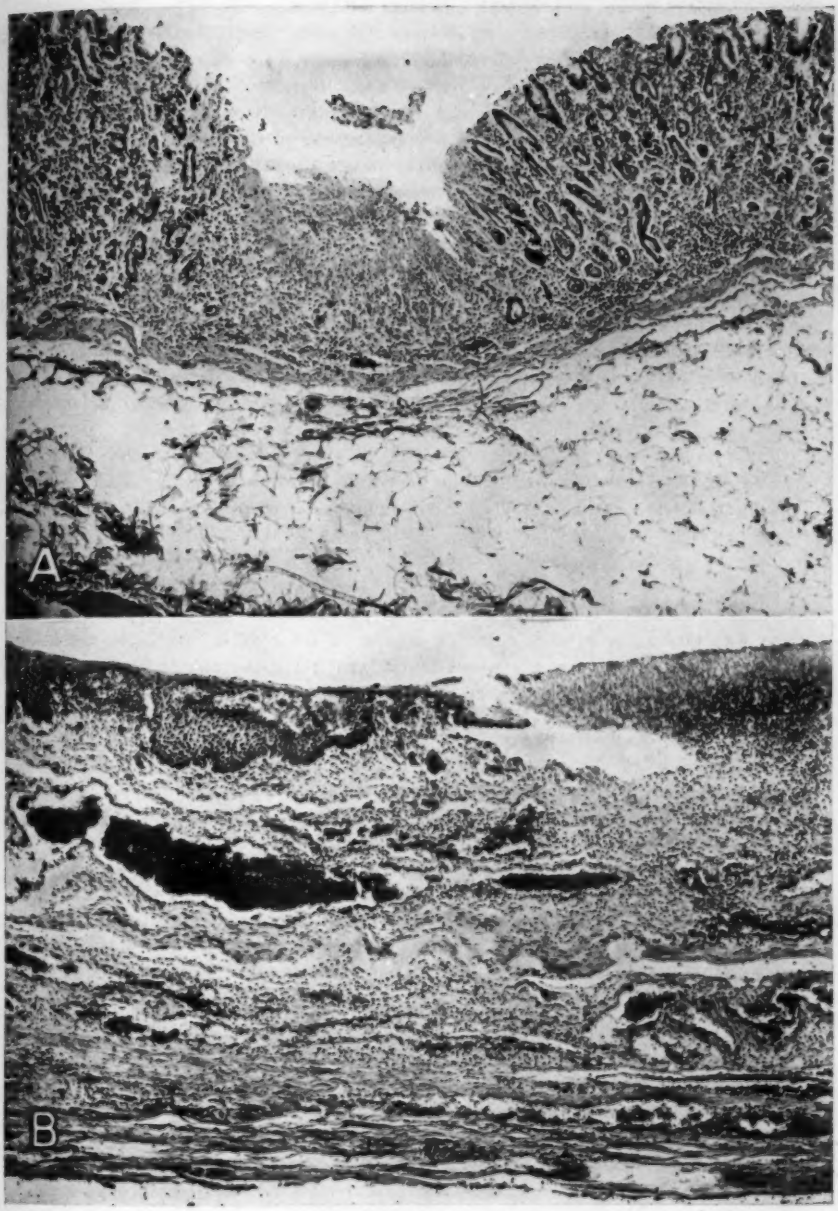
23. White, J. C.; Whitelaw, G. P.; Sweet, W. H., and Hurwitt, E. S.: Ann. Surg. **107**:287, 1938.

24. Ask-Upmark, E.: Acta psychiat. et neurol., 1935, supp. 6, p. 1.

necropsy revealed the presence of acute gastric or duodenal ulcerations or erosions either alone, together or in combination with acute ulcerative or diphtheritic esophagitis, enteritis or colitis. In selecting these cases we did not include any in which there was present a diffuse vascular disease, such as periarteritis nodosa, malignant nephrosclerosis or chronic glomerulonephritis with uremia. Further, we did not include any case in which an operation had been performed on the stomach or duodenum. We also excluded from our consideration cases of definite sepsis and cases in which chronic gastric or duodenal lesions were present. There were clinical and pathologic data for study in 37 cases, but the histologic material was necessarily scant and of value in only 18 cases. Four cases are reported in detail to illustrate our findings.

CASE 1.—H. E., a 49 year old man, was admitted to the medical service of Dr. George Baehr with a four months' history of a gnawing pain in the left upper quadrant of the abdomen, postprandial diffuse abdominal cramps, and anorexia, accompanied by infrequent nausea but no vomiting. During this period there had been noted also progressive weakness, increasing dyspnea and a loss of 14 pounds (6.4 Kg.) in weight. A guaiac test of the stool was 3 plus; the hemoglobin content was 80 per cent; the blood pressure was 130 systolic and 90 diastolic. An electrocardiogram showed changes indicative of left ventricular hypertrophy with myocardial damage and intraventricular block. A barium sulfate enema revealed an irregular stenosing neoplasm in the transverse colon. The patient was transferred to the surgical service of Dr. Ralph Colp, where an exploratory laparotomy revealed inoperable carcinoma of the hepatic flexure, with metastases to the liver and the tail of the pancreas. Palliative ileosigmoidostomy was performed, and the ileum was divided and sutured about 6 inches (15 cm.) proximal to the ileocecal valve. The first day after operation the patient's temperature rose as high as 104 F., and generalized abdominal tenderness developed. His blood pressure was 168 systolic and 70 diastolic. Coarse rales appeared in the lower lobes of both lungs. On the fourth postoperative day the abdomen became markedly distended, and drainage from the Levin tube was noted to have a fecal odor. The patient became cyanotic. An electrocardiogram taken at this time showed only slight changes. He rallied slightly for a short time under oxygen therapy; then he gradually became more cyanotic and died on the sixth postoperative day.

Postmortem Observations.—The peritoneal cavity contained about 450 cc. of dirty brown, turbid fluid. The small intestinal loops were irregularly agglutinated and covered by greenish friable material. All the operative suture lines were intact. The small intestine proximal to the anastomosis was enormously distended. The serosa of the jejunum showed occasional areas of reddening with pinhead-sized hemorrhagic dots. The stomach was distended. The mucosa of the fundus and along the magenstrasse was reddened, and there were a number of hemorrhagic dots and pinhead-sized erosions over the posterior wall; a few also were present along the upper part of the magenstrasse. On the anterior wall of the fundus there were innumerable superficial erosions from 2 to 4 mm. in diameter, with a moderate number ranging up to 6 to 7 mm., the latter being quite red. The duodenum was distended. About a foot from the duodenojejunal flexure the mucosa became slightly reddened and covered, especially at the base of the rugae,



A, focal necrosis in the mucosa of the fundus of the stomach extending down to the muscularis mucosae (case 1). In addition, interglandular edema is shown, most prominent near the free border of the mucosa. *B*, section through the edge of an esophageal erosion, showing the sharp line of demarcation as well as the pronounced vascular engorgement and submucosal edema (case 4).

by a pseudomembrane of yellowish granular friable material. This change was at first somewhat scattered but soon became extensive, with the necrotic material reaching to the apex of the folds. In places there were necroses of the apexes of the folds. There were two such areas about 6 inches (15 cm.) long with an intervening area of mucosa of about equal length where the jejunum showed nothing remarkable. About 6 inches beyond the end of the second necrotic stretch there was a small area of reddening of the mucosa with erosion of the apexes of the folds. The rest of the gastrointestinal tract was without other significant changes except for the carcinoma located at the hepatic flexure of the colon. Additional findings included fibrosarcoma of the tail of the pancreas and hilus of the spleen, with metastases to the liver, and bronchopneumonia.

Microscopic examination of the stomach through an erosion showed primarily mucosal alterations. There was a focal area of necrosis extending down to the muscularis mucosae, in which the glands were destroyed and the tissue consisted of an eosinophilic mass with nuclear débris and red blood cells. Close to this, in the mucosa, was a focal area of hemorrhage. In addition, the interglandular stroma appeared widened in places, with some edema fluid present. This was especially noticeable near the free borders of the glands. The underlying submucosa showed no significant changes. The involved portion of the jejunum showed striking dilatation of the vessels of the submucosa, where venous and capillary congestion was also striking. Some edema was present. The vessels between the muscle bundles in the muscle coats were blood filled. There were, in addition, scattered areas of necrosis, confined mainly to superficial portions of the mucosa. In focal areas, however, this necrosis extended through the mucosa, and in one area there was ulceration extending to the depths of the muscle layer. In the areas where the ulceration was extensive, there was a considerable cellular reaction, primarily of lymphocytic cells.

Case 2.—S. K., a 70 year old woman, was admitted to the medical service of Dr. George Baehr. She was known to have had hypertension for two years, with progressive dyspnea, precordial pain and weakness. For three days before admission she had experienced anorexia, headache, vertigo, insomnia and rapid respirations. On admission she was dehydrated, with poor turgor of the skin and softness of the eyeballs; the blood pressure was 150 systolic and 70 diastolic, and the hemoglobin content 107 per cent. She showed moderate cyanosis and marked peripheral sclerosis. Her diabetes was controlled by a diet of 80 Gm. of carbohydrate, 60 Gm. of fat and 40 Gm. of protein with the daily doses of insulin of 10-0-10 units and the acetonuria present on admission disappeared. Despite this, her temperature rose to 105.2 F.; intravenous administration of fluids was of no avail; she became progressively more dehydrated and died three days after admission. A 25 per cent solution of pyridine betacarbonic acid diethylamide (coramine), 3 cc., and epinephrine hydrochloride 7 minims (0.061 cc.) were given as a last resort.

Postmortem Observations.—The peritoneal surfaces were everywhere gray, smooth and glistening. There was no free fluid. The esophagus showed some mucosal congestion, and for about 5 cm. above the cardia it was greenish black along the longitudinal folds. The stomach was markedly dilated. It was studded with numerous slightly depressed round black areas, varying from 1 to 2 mm. in diameter. About 3 cm. below the cardia there were two slightly depressed mucosal defects, round in outline, each 4 mm. in diameter, with a smooth gray base and surrounded by slightly hemorrhagic mucosa. In the antrum, immediately above the pylorus, there were several mucosal defects as described for the cardia, but

slightly larger, some of which had a hemorrhagic base. The first portion of the duodenum was intensely congested. There were several black areas, serpiginous in outline, varying from 0.5 to 2.5 cm. in diameter. In addition, there were some mucosal defects as described for the stomach. The rest of the gastrointestinal tract was without significant change.

Microscopic examination of the duodenum showed widespread necrosis of the mucosa with destruction of glands and diffuse leukocytic infiltration. It showed a submucosa markedly widened by deposition of fibrin and edema fluid, hemorrhage and leukocytic infiltration. The muscular layers were substantially without change except for congestion of the smaller vessels, more intense toward the submucosa. One larger vein in the muscularis showed an organized mural thrombus. In the area where the mucosa was completely necrotic and the submucosa showed the aforementioned changes, Brunner's glands were uninvolved. Sections taken from the stomach showed severe postmortem changes. In addition, there were focal areas of hemorrhage in the mucosa with interglandular edema fluid.

Case 3.—S. G., a 36 year old man, had been in the hospital before, having been discharged about one month prior to this admission, at which time a diagnosis of intramedullary cord disease was made. He reentered the neurologic service of Dr. Israel Straus because of progression of his symptoms, particularly weakness of the left arm. On his previous admission it was determined that he had no block, and the spinal fluid showed no increase in protein. No gross tumor or disease of the cord was found on exploratory laminectomy of the cervical region. An infection of the wound developed. About four weeks after operation his temperature rose steadily, and meningeal signs were manifest. He died with a picture of meningitis and hyperpyrexia, having been febrile from the day of operation to death.

Postmortem Observations.—The peritoneal surfaces were smooth and glistening, and the cavity was free from fluid. The intestines were moderately distended. The stomach was slightly congested. On the posterior surface of the duodenum, about 3 cm. from the pyloric ring there was a circular fiery red area, about 1 cm. in diameter, slightly depressed below the surface. The margins of the ulcer were firmer than the surrounding tissue. About 2 cm. below this, on the posterior surface of the duodenum, there was another fiery red depressed area, about 3 cm. in diameter. In this case, however, there was no evident marginal induration. A few centimeters below this, at the level of the papilla of Vater, there was a small fiery red area running transversely to the duodenum along the summit of one of the mucosal folds. There was no area of induration. The remainder of the gastrointestinal tract showed no significant changes. The primary neurologic lesion was found to be a parietal dural endothelioma on the right.

Microscopic examination of the duodenal lesion showed a focal area of mucosal necrosis with underlying hemorrhage and focal polymorphonuclear reaction. Brunner's glands in this region were partially maintained and only focally destroyed. The submucosa in this area was markedly edematous, without congestion. Occasional small vessels contained eosinophilic granular masses. The external layers were unchanged. There was no evidence of chronic inflammatory reaction.

Case 4.—D. de C., a 40 year old Italian laborer, was admitted to the surgical service of Dr. Richard Lewisohn with a history of onset of generalized abdominal cramps two weeks previously with intermittent vomiting. The day after onset the pain localized in the right lower quadrant of the abdomen. There was low

grade fever for a week before admission. The patient did not appear acutely ill. The abdomen was soft. There was a mass the size of a sausage in the right lower quadrant of the abdomen, slightly tender, and there was slight resistance in the right subcostal region anteriorly. The blood pressure was 110 systolic and 70 diastolic. The white blood cell count was 9,300, with 70 per cent polymorphonuclears. The hemoglobin content was 92 per cent. Two days after admission laparotomy was performed with the patient under spinal anesthesia, and a retrocecal and retrocolic abscess was opened and drained. It contained two fecaliths. Postoperatively the patient was persistently distended in spite of a Levin and a rectal tube. On the fourth day after operation he eviscerated subcutaneously, and the bowel had to be reduced. Thereafter he did poorly. The pulse rate remained almost constantly at 120 or over, the temperature gradually mounted to 105.4 F., and death occurred three days later, with signs of spreading bronchopneumonia and diffuse peritonitis.

Postmortem Observations.—The abdomen was distended. No jaundice, petechiae or edema was noted. The appendical drains entered a walled-off abscess cavity, about 7 cm. in diameter, containing about 20 cc. of fairly thin, slightly sanguineous yellow fluid. The small intestine was markedly distended; its surface was slightly dulled and covered with occasional deposits of fibrin. There was a small amount of slightly turbid yellow fluid in the abdominal cavity. The esophagus showed in its lower half large areas of mucosal denudation and areas where the mucosa was replaced by longitudinal layers of dull yellow membranous material. The cardiac portion of the stomach showed, especially along the lesser curvature, irregular areas where the mucosal surface was dulled and granular. Near the cardioesophageal junction were small zones of mucosal roughening with small mottled purple-brown polypoid excrescences, measuring 0.2 to 0.5 cm. The remainder of the stomach was unchanged. The small intestines were markedly distended and contained a large quantity of thin brownish fluid. The mucosa was smooth, pale and glistening. The cecum was distended. The appendix was inflamed and at the tip there was a perforation leading into the abscess cavity described. The large intestines and the rectum were unchanged.

Microscopic examination of the esophageal lesion showed edema and enormous congestion extending through the entire submucosa into the mucosa, with focal hemorrhages in the mucosa. The muscularis also showed marked vascular congestion. There were focal areas in which the squamous epithelium was replaced by a necrotic fibrinopurulent pseudomembrane, beneath which the capillaries contained hyaline thrombi. The cardiac glands were uninvolved.

The histologic observations described in the esophageal and duodenal lesions are strikingly similar to those previously described in our study of acute postoperative enterocolitis. The earliest lesion appears to consist in marked distention of the capillaries and venules, first in the submucosa and subsequently in the mucosa. Next one finds focal hemorrhages in relation to distended vessels, then focal mucosal necroses. These spread and fuse, and where they are extensive they may penetrate the deeper layers of the wall and be accompanied by inflammatory cellular reactions. Hyaline thrombi are irregularly observed in the smaller vessels. Focal sparing of the gastric glands in involved areas of the lower portion of the esophagus and of Brunner's glands in the duodenum was noted.

The histologic pictures of the gastric erosions and ulcerations appear to be different. The earliest changes are observed in the mucosa, where vascular congestion and subsequent focal hemorrhage, initially more prominent toward the base of the glands, near the muscularis mucosae, are seen. Widening of the interglandular stroma is observed, and the presence of mucosal edema. Focal mucosal necroses appear, unaccompanied by any significant cellular reaction. More extensive necroses may penetrate the deeper layers of the wall and be accompanied by an inflammatory cellular reaction. With the early or superficial lesions, the submucosa, muscularis and serosal layer may be without recognizable changes. The observation that the gastric changes begin in the mucosa itself may be attributed to the fact that the most peripherally located arterial plexus in the stomach lies in the mucosa and just within the muscularis mucosa (Djørup[25]), while in the esophagus and in the small and large intestines this plexus is located in the submucosa (Patzelt[26]).

COMMENT

The focal destruction of the mucosa which we previously noted in the ulcerative enterocolitides is observed to involve the esophagus, stomach and duodenum in the cases presented here. As in the ulcerative enterocolitides, these lesions are here found in association with a wide variety of "primary" conditions. Among the latter are acute appendicitis with peritonitis, thoracotomy for pulmonary gangrene and for pulmonary abscess, cholecystectomy with pericholecystic abscess, suprapubic prostatectomy, diabetic acidosis, laminectomy with meningitis, and other conditions. In addition to the "primary" conditions which occurred in our own series we should note that identical lesions have been observed in the newborn in association with birth trauma, as well as in persons suffering from burns or freezing or from intracranial operations (see earlier comment on this).

As indicated, this wide variety of primary conditions have in common the factor of shock, whether this is due to peritonitis, diabetic acidosis, hemorrhage, trauma or operation. The physiologic responses to the process of shock regardless of its cause have been discussed in our previous paper. We may briefly summarize them as follows: The vascular response to the diminution in blood volume which characterizes shock consists in arteriolar contraction. The duration of this reaction depends on the duration of the application of the shocking process and on the accumulation of local metabolites, which have the property of causing vasodilatation. The anoxemia resulting from the vasoconstric-

25. Djørup, F.: Ztschr. f. Anat. **64**:279, 1922.

26. Patzelt, V., in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1936, vol. 3.

tion and the associated drop of the gradient of pressure within the capillaries lead to an increase in the permeability of the capillary endothelium. As a consequence there is transudation of plasma into the tissue stroma, and if the process continues cellular components also pass through the capillary walls into the surrounding tissues. This is especially true of the red blood cells, and we thus obtain the punctiform hemorrhages already noted. These are considered, in the absence of any morphologic change in the vessel walls, to be the result of diapedesis. The process eventually terminates in necrosis of the tissue in the field of the blood supply of the affected vessels.

In this manner one may easily understand the occurrence of focal necroses in such a wide variety of clinical conditions.

SUMMARY

We have reported in detail 4 cases of ulcerative lesions in the esophagus, stomach and duodenum which appeared postoperatively or subsequent to diabetic acidosis. These are representative of the group of 47 cases which were reviewed in necropsy experience during a period of about ten years. In selecting these cases we omitted any in which a systemic disease might have caused the lesions, i. e., diffuse vascular disease, such as malignant sclerosis, chronic glomerulonephritis, periarteritis nodosa or sepsis. We also omitted cases in which an operative procedure had been performed on the stomach or in which a chronic ulcer of the stomach or duodenum was present.

A reconstruction of the sequence of histologic events on the basis of our observations indicates that the earliest change consists of marked distention in capillaries and venules, which is seen in the mucosa in the stomach and in the submucosa in the esophagus and duodenum. An anatomic explanation for this is offered. Edema fluid accumulates, and focal hemorrhages are frequently observed. These are due to the increase in permeability of the capillaries resulting from the stasis which is the consequence of the arteriolar constriction coming on in response to shock. The further progress of the process results in necrosis of the tissues in the foci involved.

CONCLUSION

From a study of a series of cases of acute ulcerative lesions of the esophagus, stomach and duodenum and from a review of similar cases in the literature, we have concluded that the vasomotor mechanisms which constitute the response to shock produce these lesions.

ATROPHY OF THE CREMASTER MUSCLE

JOHN R. McDONALD, M.D.
AND
CHARLES W. MAYO, M.D.
ROCHESTER, MINN.

The incidence of diseases which produce anatomic changes in striated skeletal muscle is relatively low. It was with this in mind that a search was made for a normal cremaster muscle to compare with a hypertrophied one. It was felt that it would be a simple matter to demonstrate a normal muscle. On the contrary, it was found necessary to examine many specimens before a cremaster muscle was found which had a normal gross and histologic appearance.

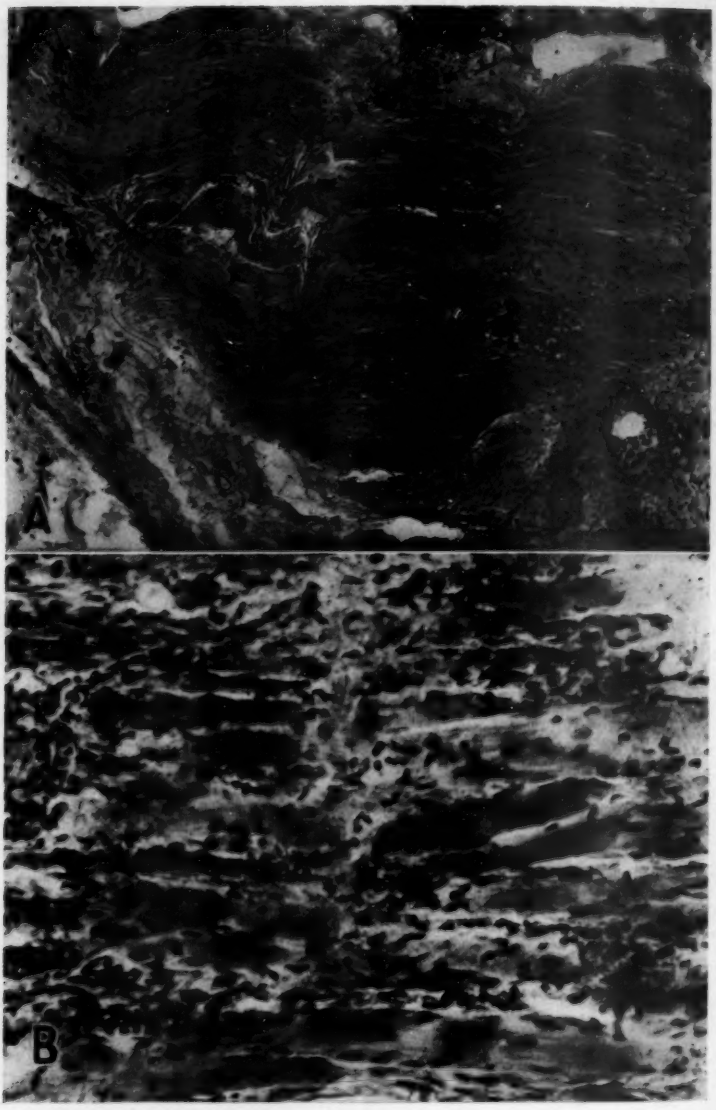
Three groups of specimens were examined in an attempt to determine the frequency as well as the possible cause of the changes that were found. The first group was picked at random from spermatic cords which had been removed together with the corresponding testes in the course of surgical repair of scrotal hernias. The second group was chosen from spermatic cords removed together with the corresponding testes in surgical treatment for testicular neoplasms. The third group consisted of spermatic cords obtained at necropsies.

On each specimen a number of sections were made across the entire thickness of the spermatic cord at a distance of at least 2 cm. above the epididymis. The sections were stained with hematoxylin and eosin. In addition, when the cremaster muscle could be identified grossly, longitudinal sections were made, mounted, and stained with hematoxylin and eosin. The sections were examined microscopically, and any variation from normal was noted.

In none of the spermatic cords examined was there hypertrophy. A total of 80 spermatic cords from different patients were examined. In no case were both cords of the same patient examined.

In 42 of the 80 spermatic cords it was impossible to demonstrate any cremaster muscle even after a careful search. Of the 38 cords in which striated muscle could be shown, the muscle was normal in only 9. In the remaining 29 specimens the muscle was infiltrated by varying numbers of lymphocytes. Occasionally polymorphonuclear leukocytes could be found scattered between the muscle fibers. In addition, a prominent change was the replacement of the muscle with collagenous

From the Section on Surgical Pathology (Dr. McDonald) and the Division of Surgery (Dr. Mayo) of the Mayo Clinic.



A, cremaster muscle removed during the repair of a scrotal hernia; hematoxylin and eosin; × 100. There are multiple collections of lymphocytes with beginning fibrosis. *B*, cremaster muscle showing marked fibrosis and many lymphocytes; hematoxylin and eosin; × 220.

fibrous tissue (*A* in figure). In some cases this replacement was so marked that very few muscle fibers could be identified (*B* in figure).

Thirty-nine spermatic cords made up the first group examined, which were obtained from patients who had inguinal hernia. A cremaster muscle could be identified in only a third of these specimens. Of these, only a single muscle was normal. The remaining 12 muscles showed infiltration by lymphocytes or fibrosis or both. The patients from whom the spermatic cords in this group were obtained were older than those from whom the other two groups were obtained, many of them being over 50 years of age.

The second group comprised 26 specimens representing cases in which the testis and a portion of the spermatic cord had been removed for a testicular neoplasm. In half of these specimens it was possible to recognize a cremaster muscle. Four of these muscles were normal on microscopic examination. As a whole, the patients from whom this group were obtained were somewhat younger than those with inguinal hernia, from whom the first group were obtained.

The third group comprised 15 specimens obtained at necropsies. Striated muscle was present in all except 3 specimens of this group. Four were normal. The ages of the patients ranged from birth to 85 years.

The average age of the patients from whom the specimens were obtained in which no cremaster muscle could be found was approximately 53 years. The average age of the patients from whom the specimens were obtained in which cremaster muscle was identified but which showed fibrosis and lymphocytic infiltration was 51 years. The average age of the patients from whom specimens showing normal cremaster muscle were obtained was 30 years.

COMMENT

It appears that fibrosis of the cremaster muscle has an extensive distribution in human males. It progresses with senescence and is seen less commonly in early life. Probably it is a retrogressive phenomenon occurring in a relatively useless structure. Trauma or incarceration of the spermatic cord undoubtedly plays a role, since fibrosis or absence of the cremaster muscle was very common in those cords that came from patients with inguinal hernia.

Although the cremasteric reflex is absent in 26 per cent of newborn infants (Aronovitch[1]), it is present in all but 2.17 per cent of boys between the ages of 1 day and 10 years. In boys the reflex may be so active that the testis rises with each inspiration and falls during expira-

1. Aronovitch, G.: J. Nerv. & Ment. Dis. **64**:235, 1926.

tion (Jastrowsky [2]). Mitchell [3] and Purves-Stewart [4] observed that the cremasteric reflex is sluggish and sometimes absent in old men. Mitchell noted further that old hernias and trusses tend to lessen or destroy the cremasteric reflex. The absence or refractoriness of the cremasteric reflex in these old men can be explained entirely by the phenomenon of fibrosis and disappearance of the cremaster muscle. It is unlikely that changes in the reflex arc play an important role.

SUMMARY

The cremaster muscle frequently shows collections of lymphocytes with fibrous replacement of the muscle fibers. This change occurs most frequently in old men, especially those with inguinal hernia. The absence or diminution of the cremasteric reflex in old men can be explained by these changes in the cremaster muscle.

2. Jastrowsky, cited by Mitchell.[3]

3. Mitchell, S. W.: J. Nerv. & Ment. Dis. **4**:577, 1879.

4. Purves-Stewart, J.: The Diagnosis of Nervous Diseases, ed. 6, London, Edwin Arnold & Co., 1924.

NEW BONE FORMATION IN A PRIMARY CARCINOMA OF THE PROSTATE GLAND

L. L. ASHBURN, M.D.
Passed Assistant Surgeon, United States Public Health Service
WASHINGTON, D. C.

Carcinoma of the prostate gland with osteoplastic metastases to bone is a frequent and well known condition. However, bone in the stroma of a primary prostatic carcinoma has not previously been recorded. Nor has it been found in the soft tissue metastases of carcinoma of the prostate except for the case reported by Schmorl.[1] In this case bony-hard nodules were found in the lungs, and Schmorl interpreted the condition as osteochondrosarcoma developing in the stroma of the skeletal metastases of the prostatic carcinoma, with secondary sarcomatous and mixed deposits in the lungs. Heterotopic bone formation is not infrequent and occurs in many different organs and structures. However, it appears that bone formation in the prostate gland is extremely rare. Huggins[2] in listing the various extraskeletal locations in which bone has been found did not include the prostate.

The case of prostatic carcinoma presented in this report is unique in that a nodule of bone measuring 10 by 14 mm. was present deep in the prostate, intimately associated with the tumor cells. An additional feature of considerable interest was that in this nodule were a few foci of cartilage and one small area having the structure typical of the very rare gelatinous carcinoma of this organ.

REPORT OF CASE

Clinical History.—A white man aged 68 years was admitted to the United States Marine Hospital, Portland, Me., Dec. 21, 1936, with the usual symptoms of prostatic carcinoma of about one year's duration. He had no pyuria, hematuria or recent loss of weight.

Physical examination disclosed a moderately enlarged hard prostate with areas of stony hardness in the left side. There was considerable reduction in the size of the prostatic urethra, a small metal catheter being passed with difficulty. The residual urine measured 8 ounces (236.5 cc.), and excretion of phenolsulfonphthalein was retarded.

From the Division of Pathology, National Institute of Health.

1. Schmorl: Verhandl. d. deutsch. path. Gesellsch. **12**:89, 1908.
2. Huggins, C. B.: Arch. Surg. **22**:377, 1931.

A suprapubic cystotomy was done December 26, followed by prostatectomy Jan. 15, 1937. The large indurated prostate was enucleated with difficulty and showed two quite hard, apparently calcified areas.

Examination June 15 showed a loss of 8 pounds (3.6 Kg.) in weight. The patient was almost totally incontinent. Urinalysis showed a slight trace of albumin and few white blood cells. There was a very large hard recurrent prostatic tumor, and nodular masses extended up both sides of the pelvis, obliterating all landmarks. Treatment with roentgen rays of high voltage was begun on June 16 and continued until November 10. A total of 7,300 roentgens was given. Jan. 31, 1938, by letter, the patient reported his condition as unimproved. No further information has been obtainable up to the time of writing (December 1938).

This history was furnished by Dr. J. E. Hammond and Dr. M. B. Noyes of the United States Marine Hospital, Portland, Me.

Microscopic Examination of Prostate.—In each of two blocks there was a well circumscribed nodule showing increase in the number and size of alveoli, lined by hyperplastic and focally stratified columnar epithelium. In some areas there were fairly prominent intra-alveolar papillary projections. Marginating these nodules and in three other blocks there was moderate to marked invasion of the stroma by variably sized tubular acini, alveoli, atypical tubules and solid or multiluminate sheets of cells. Some of the alveoli showed many papillary ingrowths, the fusion of which gave a fenestrated appearance. The cells were cubic, columnar or polygonal and had ample clear or lightly oxyphilic cytoplasm and large round hyperchromatic vesicular or leptochromatic nuclei, usually without distinct nucleoli. Mitoses were infrequent. There was marked tumor invasion of the capsule and of the muscular wall of the seminal vesicles. Here the acini were generally small and numerous and were seen as deep as the base of the mucosal folds. Tumor cell groups were seen in a few lymphatics, particularly the perineural vessels.

The grossly stony nodule measured 10 by 14 mm. and was formed largely of masses and ramifying trabeculae of bone, apparently of the membranous type. The spaces between the bone lamellae frequently contained large masses of tumor cells or a few loosely disposed tumor cells set in a fibrous stroma. Occasionally these tumor cells were present just within the bone lamellae. A layer of dense fibrous tissue separated the bony nodule, at least in part, from the remainder of the gland. Tumor acini were present only in the outer margin of the nodule. There was one poorly circumscribed, irregularly shaped area, measuring 2 by 3 mm., in which tumor cells were arranged in columns or strands separated by bands of fibrillary mucin (stained red with mucicarmine and metachromatic with toluidine blue). The bands were frequently from two to three times as thick as the cell columns. This structure graded on one margin into solid tumor tissue; on the opposite side mucin was more copious and single cells were isolated. The latter margin abutted directly on atypical cartilage having large lacunae and much thready metachromatic matrix. A few cell-containing lacunae surrounded by a moderate amount of matrix occurred singly or in small groups in marginating bone. The tumor cells in this nodule were large and irregular in shape, and had quite hyperchromatic nuclei and distinct nucleoli. Few multinucleate cells were seen. Mitoses were moderately numerous, two of which occurred in cells within lacunae.

A second area, measuring 2 by 3 mm., showed larger and more numerous lacunae surrounded by a thready metachromatic matrix in which a few collagen fibrils occurred. The collagen fibrils were more numerous in the periphery of the nodule and were continuous with the surrounding fibrous tissue.

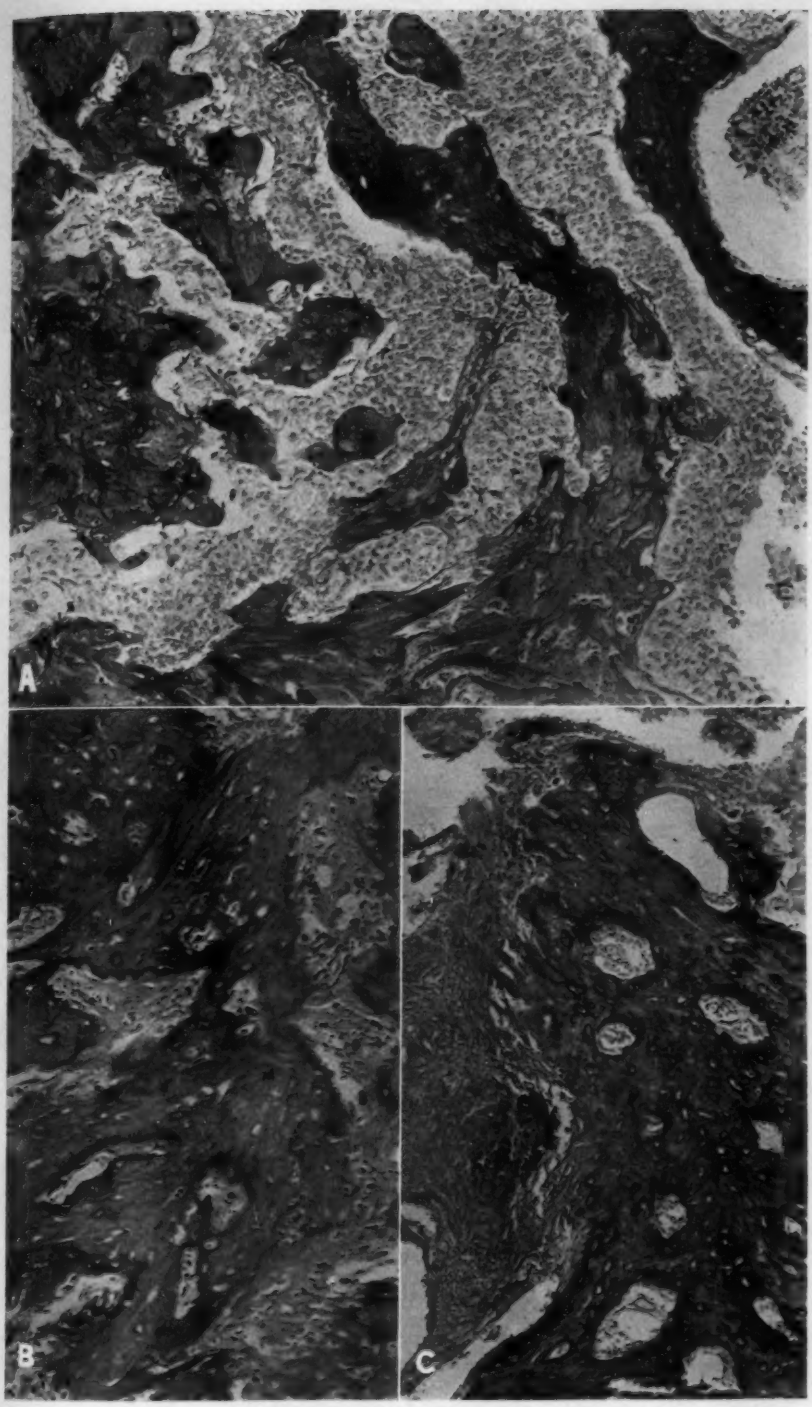


Fig. 1.—*A*, lamellae of bone separated by sheets of tumor cells. Van Gieson's stain; × 80. *B*, irregularly directed bone lamellae separated by fibrous tissue in which occasional to few tumor cells occur. Van Gieson's stain; × 80. *C*, section showing gradation from bone to fibrous tissue. Van Gieson's stain; × 80.

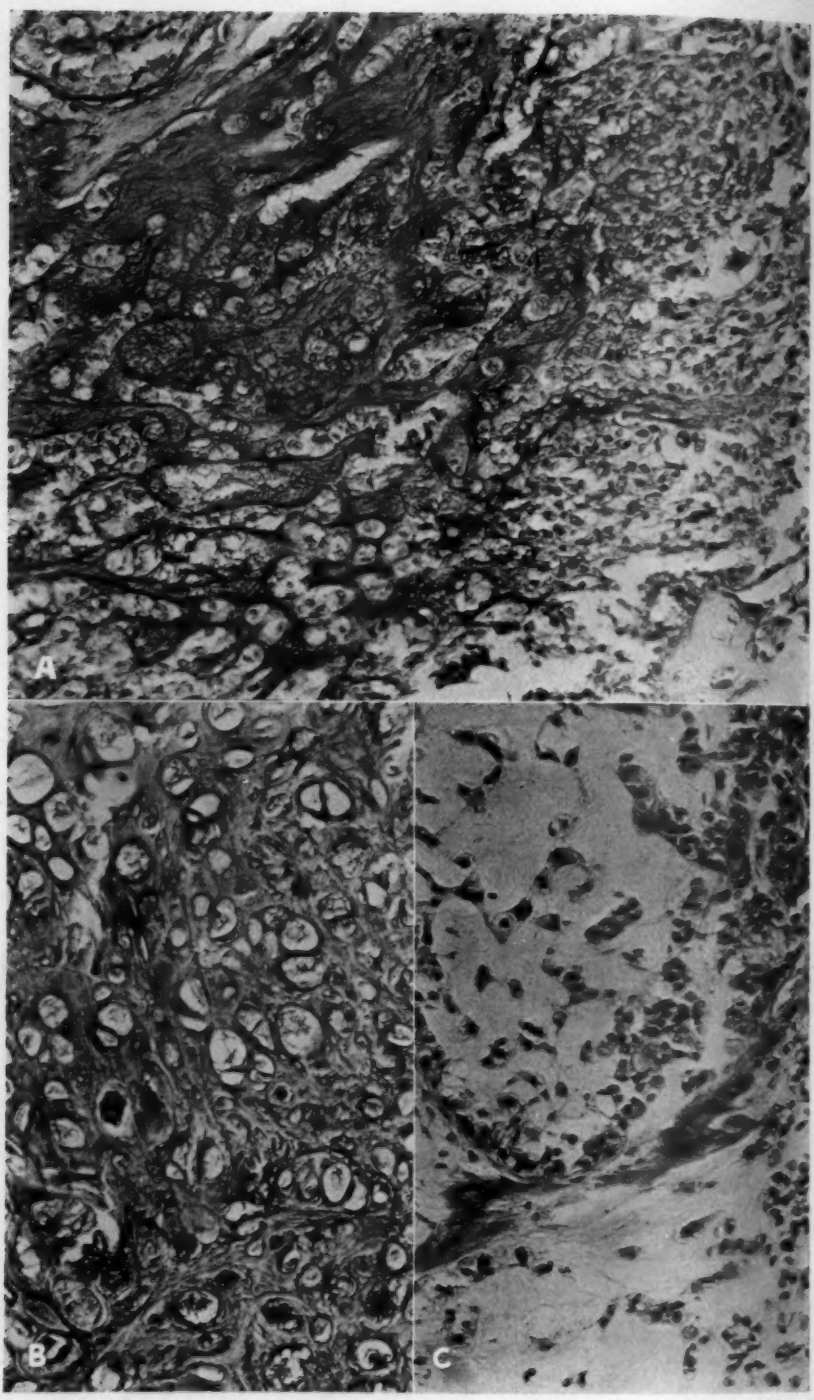


Fig. 2.—*A*, solid tumor at right; much intercellular thready mucin at left. Note the intergradation. Toluidine blue stain; × 130. *B*, area of atypical cartilage; the matrix is thready and metachromatic. Toluidine blue stain; × 130. *C*, area having the structure of gelatinous carcinoma (similar to *A*). The intercellular thready mucin is not stained. Van Gieson's stain; × 130.

COMMENT

The mechanism of bone formation in the skeletal metastases from carcinoma of the prostate has been the subject of much discussion, and numerous theories have been formulated. Von Recklinghausen [3] in 1891 gave a classic description of this condition and suggested that local congestion resulting from numerous tumor thrombi was the factor initiating the bone growth. Courvoisier [4] thought that the osteogenesis resulted from the accompanying inflammatory reaction. Assmann [5] believed that primary necrosis of bone was necessary before new bone could be formed. Quite contrary to these views was the suggestion by Axhausen [6] that the cancer cells liberated a chemical factor or stimulus which initiated the formation of bone. He did not believe that the cancer cells assumed the function of osteoblasts, a belief held by Braun,[7] Kaufmann [8] and Sasse.[9]

Recent studies on this subject have borne out the view held by Axhausen. Gutman, Sproul and Gutman [10] showed that there is increased phosphatase activity of bone at the site of osteoplastic metastases secondary to carcinoma of the prostate gland. They feel that the capacity of the tumor cells to stimulate production of bone phosphatase determines in part the osteoplastic character of the metastatic lesion. Huggins [11] showed that in dogs bladder epithelium transplanted to the sheath of the rectus abdominis muscle has the property of initiating formation of bone and that there is increased phosphatase activity [12] in these heterotopic foci of bone.

Since prostatic epithelium is closely related embryologically to bladder epithelium, it may be true, as suggested by Huggins,[2] that prostatic epithelium also has "osteogenic" properties. This assumption would serve as an attractive theory in explaining the presence of bone in the case presented here. The bone was of the membranous type, apparently being formed directly from dense fibrous tissue, and although a few

3. von Recklinghausen, F.: Die fibröse oder deformierende ostitis, die Osteomalacie und die osteoplastische Carcinose in ihren gegenseitigen Beziehungen, in Arbeiten aus dem Kaiser- und Kaiserin- Friedrich- Kinderkrankenhause in Berlin, Festschrift, Herrn Rudolph Virchow zum 70. Geburtstage, Stuttgart, Ferdinand Enke, 1891, pp. 1-89.

4. Courvoisier, W.: Das Prostatacarcinom, Inaug. Dissert., Basel, M. Werner-Riehrn, 1901.

5. Assmann, H.: Virchows Arch. f. path. Anat. **188**:32, 1907.

6. Axhausen, G.: Virchows Arch. f. path. Anat. **195**:358, 1909.

7. Braun, L.: Wien. med. Wchnschr. **46**:481, 527 and 582, 1896.

8. Kaufmann, E.: Lehrbuch der speciellen pathologischen Anatomie für Studierende und Aertze, Berlin, George Reimer, 1911, pp. 759-762.

9. Sasse, F.: Arch. f. klin. Chir. **48**:593, 1894.

10. Gutman, E. B.; Sproul, E. E., and Gutman, A. B.: Am. J. Cancer **28**:485, 1936.

11. Huggins, C. B.: Proc. Soc. Exper. Biol. & Med. **27**:349, 1930.

12. Huggins, C. B.: Biochem. J. **25**:728, 1931.

isolated cancer cells were seen in the peripheral margins of some bone lamellae, they did not appear to be assuming the function of osteoblasts. Bone cells in all lamellae were of the adult, small type. Osteoblasts palisaded against bone were not found. Since this case shows that cells capable of acting as osteoblasts are present in the prostate, the question naturally arises: Why is bone not found more often in cancerous prostates? This question cannot at present be answered. However, Huggins, McCarroll and Blocksom [13] found that the corium of the urinary bladder has an inhibiting influence on the osteogenic property of the overlying epithelium and that this inhibiting factor is not present in the outer part of the bladder wall. Huggins [2] saw no production of bone in transplants of prostatic tissue. He suggested that the intimately associated stroma, which could not be removed from the epithelium, might have an inhibiting influence similar to that of bladder mucosal corium. If the prostatic stroma does have an inhibiting influence on bone formation, this case demonstrates that it can be overcome under some conditions.

The areas in the hard nodule having the structure of gelatinous carcinoma are interesting because of the rarity of this type of carcinoma of the prostate, Boyd [14] reported such a case, and more recently Klissurow [15] made a detailed study of a case in which carcinoma of the prostate gland of the usual type was present except in the left lobe. In this lobe it was of the gelatinous type. Considering the embryology of the gland and the fact that Pilcher [16] demonstrated the presence of variable amounts of mucus in a large percentage of cases of carcinoma of the prostate gland, it is surprising that more cases of frank gelatinous carcinoma are not found.

The relationship of the area of mucous carcinoma to the adjacent cartilage is interesting but difficult to understand. For the greater part, there was abrupt change from one type of tissue to the other. However, at one point there appeared to be a transition, and the cells within the lacunae appeared quite similar to the cancer cells lying between the strands of mucus. Also, a few of the cartilage cells were seen in mitosis.

SUMMARY

A case of carcinoma of the prostate gland is presented in which a stony-hard nodule was present composed mainly of bone but showing also foci of cartilage and one area having the structure of gelatinous carcinoma. The possible significance of the bone formation is discussed.

13. Huggins, C. B.; McCarroll, H. R., and Blocksom, B. H., Jr.: Arch. Surg. **32**:915, 1936.

14. Boyd, S.: Tr. Path. Soc. London **33**:200, 1882.

15. Klissurow, A.: Virchows Arch. f. path. Anat. **268**:515, 1928.

16. Pilcher, F., Jr.: Am. J. Clin. Path. **8**:366, 1938.

DISSEMINATED ENCEPHALOMYELITIS OF THE DOG

LESTER S. KING, M.D.
PRINCETON, N. J.

In 1930 Perdrau and Pugh,[1] in England, called attention to demyelinizing lesions in the central nervous system of the dog suffering from what they called "the 'nervous form of canine distemper.'" They found that 4 of 14 animals showed this reaction. The demyelination, when present, was observed chiefly in the cerebellar peduncles or folia and was generally accompanied by heavily cuffed blood vessels.

At the same time, but independently, Posrednik[2] described similar lesions. In 9 cases of experimentally induced distemper with a greater or a less degree of encephalitis, he observed a very slight degree of demyelination in 1 instance. But in 3 cases of the disease occurring naturally the loss of myelin was more pronounced, especially in the cerebellum, medulla and pons. Subsequently Marinesco, Draganesco and Stroesco,[3] as well as Peters and Yamagiwa,[4] gave detailed descriptions of the histologic changes in cases of distemper and confirmed the observation of loss of myelin in many of the lesions.

The literature on distemper is extensive. This virus-induced disease may attack the nervous system to produce nervous manifestations but does so inconstantly. Apart from the loss of myelin which is sometimes observed, the principal features described by the older investigators are those of encephalitis and meningoencephalitis, namely: perivascular accumulation of lymphocytes; inflammatory infiltrations involving the parenchyma and the meninges, sometimes very intensive; occasional perivascular hemorrhage; endothelial proliferation; variable degrees of degenerative neuronal damage; changes in the glial apparatus, both proliferative and regressive. The intracellular inclusions, first described thirty years ago, have recently been studied and the relevant literature reviewed by De Monbreun.[5] He found the inclusions abundantly present

From the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research.

1. Perdrau, J. R., and Pugh, L. P.: J. Path. & Bact. **33**:79, 1930.

2. Posrednik, F. I.: Ztschr. f. Infektionskr. **38**:135, 1930.

3. Marinesco, G.; Draganesco, S., and Stroesco, G.: Ann. Inst. Pasteur **51**: 215, 1933.

4. Peters, G., and Yamagiwa, S.: Arch. f. Tierh. **70**:138, 1935.

5. De Monbreun, W. A.: Am. J. Path. **13**:187, 1937.

in the brain as well as in other diseased tissues. Many authors, however, have not been able to find inclusions in their cases, although, among recent writers on the neuropathology of the disease, excellent illustrations have been given by Gallego [6] and by Marinesco, Draganesco and Stroesco,[3] as well as by De Monbreun.[5]

The observations mentioned are those which occur when the animal shows clinically involvement of the nervous system. But Cerletti [7] described a series of dogs suffering from clinical distemper without nervous manifestations in which, nevertheless, inflammatory and toxi-degenerative lesions of the nervous system were present. This observation has been confirmed by Roman and Lapp.[8]

Although the foreign literature contains numerous studies on disseminated encephalomyelitis of the dog and its relation to distemper, little attention has been paid to the subject in this country. No observations have been made on the problem of demyelination. The following report of a case is consequently of some neuropathologic interest.

CLINICAL HISTORY

The dog reported on here was sent to this laboratory by Dr. F. A. Zucker, of Roselle, N. J.

The available history is rather sketchy. The animal was a pointer bitch, about 4 years old, first taken ill about three months previously. The original symptoms are not on record, but the animal was first treated by a veterinarian for tapeworm. A second veterinarian diagnosed brain fever. When seen by Dr. Zucker, the animal showed spasticity, stiffness of gait, with clonic movements of the forelegs, ataxia and mental impairment. The pupils did not react to light, and there was no response to pinprick over the posterior portion of the body. There was slight improvement for a few days, but then the animal became rapidly worse and was sent to this laboratory for examination.

When seen by me, the animal was in a recumbent position, lying on the right side, in marked distress. There were marked decubitus ulcers on the right shoulder and elbow. The hindlegs were flexed and completely paralyzed. Moderate contractures were present. The right foreleg was in rigid extension and strongly resistant to any passive movement. The left foreleg was only moderately spastic. No tendon reflexes were elicitable. The sensory deficit was marked, although no sharp level could be detected. Pinching and mildly painful stimuli applied to the hindlegs and trunk evoked no response. Some sensation apparently remained in the forelegs and head. Pupillary and corneal reflexes were present.

The animal was killed by chloroform anesthesia. During the struggle only the left foreleg and the head were spontaneously moved. During narcosis the spastic right foreleg became flaccid. The hindlegs showed only moderate relaxation due to the contractures.

The general autopsy showed only severe hemorrhagic cystitis. The bladder was not dilated, but the wall was markedly thickened, and the mucosa showed

6. Gallego, A.: Ztschr. f. Infektionskr. **34**:38, 1928.
7. Cerletti, U.: Ztschr. f. d. ges. Neurol. u. Psychiat. **9**:520, 1912.
8. Roman, B., and Lapp, C. M.: Bull. Buffalo Gen. Hosp. **3**:40, 1925.

inflammation, hemorrhage and necrosis. There were no other features of significance. The involvement of the bladder was undoubtedly secondary to the injury of the spinal cord.

With regard to the nervous system, in gross the most marked changes were in the spinal cord, which in portions was very soft. On section cavities were visible in some regions around the central canal. The brain showed no gross abnormalities.

The brain and cord were fixed in 10 per cent formaldehyde; pyroxylin, paraffin and frozen sections were made of representative regions.[8a] Hematoxylin-eosin, thionine, iron-hematoxylin, Van Gieson, scarlet red, Weil, silver carbonate, Bodian[8b] and Anderson[8c] staining methods were used.

MICROSCOPIC OBSERVATIONS

The changes in the nervous system are primarily inflammatory, although varying in intensity. The diseased areas are scattered throughout the entire neuraxis, with the greatest intensity in the spinal cord. The lesions in the cord are not confluent but scattered throughout its length. Some lesions, however, extend over several segments.

Spinal Cord.—A typical lesion is a fairly well delimited focus occurring in either the gray or the white substance but with greater frequency in the latter. Figure 1 *A* illustrates such a lesion. The blood vessels stand out because of the perivascular infiltration by lymphocytes, plasma cells and more undifferentiated mononuclear cells. Under higher power the endothelium may, in places, be seen to be proliferated. Between and around the blood vessels the tissue is rarefied, presenting a lacunar appearance, but axis-cylinders persist in large numbers. Apart from the sheaths of blood vessels there is little or no increase in cellularity in the damaged area. Although some progressively altered glial forms may be observed, there are no gitter cells in this particular lesion.

There are several variations on this picture, each of which presents its own significant features. Two fields from a single section illustrate different pathologic processes. In figure 1 *B* there is shown a very intense cellular reaction. The larger blood vessels (outside the field of this photomicrograph) are very heavily cuffed, but in the particular field shown the infiltration of the tissue by plasma cells is noteworthy. Gitter cells are present in moderate numbers, as well as other microglial forms. Occasional polymorphonuclear leukocytes are also scattered through the tissue. The lacunae in the tissue may, to a certain extent, be seen in process of formation, but the cellular infiltration is so heavy that details of the tissue are obscured. This is considered to be an

8a. The Weil staining method is described in McClung, C. E.: Handbook of Microscopical Technique, New York, Paul B. Hoeber, Inc., 1937, pp. 474.

8b. Bodian, D.: Anat. Rec. **65**:89, 1936.

8c. Anderson, J.: How to Stain the Nervous System, Edinburgh, E. & S. Livingstone, 1929, p. 80.

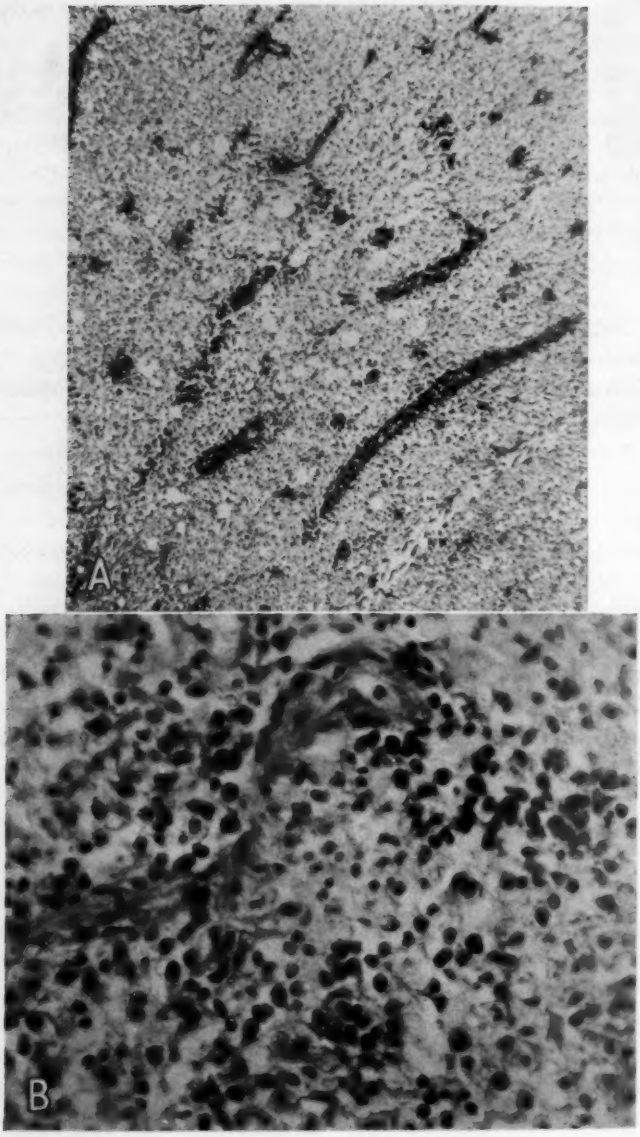


Fig. 1.—*A*, spinal cord, anterior-lateral portion. There are moderately intense perivascular infiltration and proliferation of vascular endothelium. The cribrated appearance of the white matter should be noted. On the extreme lower right, the myelin is essentially normal. Hematoxylin-eosin stain; × 61.5. *B*, spinal cord, showing infiltration of the parenchyma with plasma cells and occasional polymorphonuclear leukocytes. Hematoxylin-eosin stain; × 431.2.

acute stage of the process shown in figure 1 *A*. On the other hand, elsewhere the état criblé may be very marked, even more than in figure 1 *A*, and yet the interstitial reactions may be entirely negligible.

A quite different picture is shown in figure 2 *A*. Here there is practically complete softening, involving the anterior horn and adjacent white matter. The reactive cells are almost exclusively compound granular corpuscles, and these are not numerous. Most unusual, however, is the excellent state of preservation of the anterior horn cells in the midst of the tissue débris. The nuclear membranes are hyperchromatic, but the general cellular appearance is excellent. There are a few neurons that show degenerative changes, but they are exceptional. However, in other areas, in other sections of the cord, a similar type of damage of the tissue has destroyed the neurons as well.

Sections of the spinal cord stained for myelin show expected changes. Figure 2 *B*, of the cervical region, displays different degrees of demyelination, in accordance with the cellular picture. The regions of spongy vacuolation and rarefaction, with moderate perivascular reaction, show considerable diminution in the quantity of myelin but not total loss. Other areas show practically complete destruction of myelin. In the center there is cavitation, due to complete softening. Many of the gitter cells contain hematoxylin-staining masses of myelin, testifying to the acuteness of the process. Longitudinal sections of the cord (fig. 2 *C*) show an irregular margin as well as the usual beadings and swellings attendant on myelin disintegration.

An instructive field is illustrated in figure 2 *D*, taken from the lateral column of a cross section of the thoracic portion of the cord. Numerous punched-out areas of demyelination are present in otherwise well preserved tissue. Closer examination shows that all of these areas are heavily cuffed blood vessels, where the perivascular accumulation of cells has choked out the myelin. Some of the inflammatory cells contain minute droplets of myelin. The intervening tissue appears relatively normal. This type of demyelination seems to be in a different category from that previously illustrated. It is more comparable to the strictly focal demyelination sometimes seen after hemorrhagic encephalitis[9] rather than to the diffuse damage of tissue seen in figure 1 and figures 2 *A*, *B* and *C*. It is very doubtful that this perivascular type would develop into the diffuse forms, in which the tissue as a whole appears injured. The pathogenic factors in the two forms appear to be distinct.

Sections of the spinal cord stained for fat confirm the observations on the sections stained with hematoxylin and eosin. The amount of neutral fat, although in some areas fairly considerable, is on the whole much

9. Baker, A. B.: Am. J. Path. **11**:185, 1935. Russell, D. S.: J. Path. & Bact. **45**:357, 1937. Dobbs, R. H., and de Saram, G. S. W.: ibid. **46**:437, 1938.

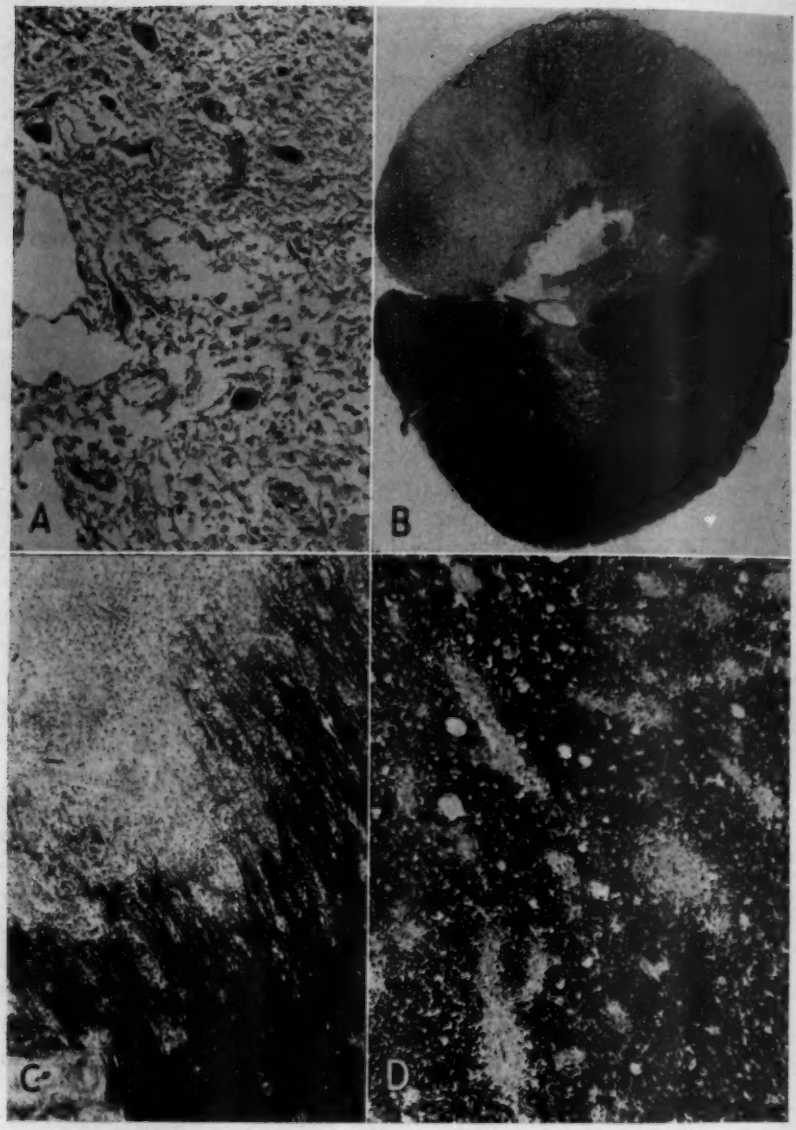


Fig. 2.—*A*, spinal cord. There is almost complete softening of part of the anterior horn and adjacent white matter. The reactive cells are almost exclusively compound granular corpuscles. The excelient preservation of the ganglion cells in the midst of the tissue débris should be noted. Hematoxylin-eosin stain; × 85.8. *B*, spinal cord, showing partial and complete demyelination. The état criblé is readily apparent. Weil stain; × 9.4. *C*, spinal cord, longitudinal section, illustrating the sharp margin of the demyelinating process. Much hematoxylin-staining material is still present in the demyelinized area. Weil stain; × 34.5. *D*, transverse section of spinal cord, showing perivascular demyelination consequent to intense perivascular infiltration. There is much phagocytosed myelin in the perivascular spaces. Weil stain; × 72.7.

less than would be expected, certainly incomparably less than would be seen in so-called acute multiple sclerosis of comparable duration. The gitter cells, as has already been noticed, are not very numerous except in certain areas of almost total softening. The Herxheimer stain shows many cells, both in the perivascular sheaths and free in the tissue, which contain a few small globules of neutral fat. The heavily crammed compound granular corpuscles are present in certain areas, but where the infiltration of the tissue is heaviest they are few.

The axis-cylinders are invariably better preserved than the myelin. Even where the destruction of tissue appears most complete, axis-cylinders persist, though distorted and damaged. In other areas they are preserved in considerable numbers. All types of degenerative changes, such as varicosities, torpedoes, swellings, splittings, fibrillary ball formations, loops and the like, are abundantly in evidence.

Repair has taken place to a certain extent, but instead of gliosis (i. e., proliferation of fibrous astrocytes and their fibers) chiefly the connective tissue is involved. Figure 3 *A* illustrates a rather marked degree of this process. For comparison with the connective tissue reaction in multiple and diffuse sclerosis, reference may be made to a previous paper.[10]

Medulla and Higher Levels.—The pathologic changes differ from those of the preceding description chiefly in degree. In general they are not so severe as those in the spinal cord.

In the medulla and pons there are scattered lesions involving the cerebellar peduncles, various scattered areas at the periphery and less frequent areas in the more central portions. Figure 3 *B* illustrates the degree of damage in the pons. In general there is fairly heavy perivascular cuffing with plasma cells and lymphocytes. Rarefaction of tissue and lacunar degeneration are common, but white blood cells are not frequent in the parenchyma. Numerous compound granular corpuscles are present. Apart from the discrete lesions the tissue is entirely normal.

In the cerebellum too the process is similar. The damaged areas are fairly well circumscribed and scattered through the cerebellar white matter. The perivascular infiltration is often quite dense, and infiltration of the tissue by inflammatory cells may be well marked. However, it is nowhere as severe as in the spinal cord. In the cerebellum itself, in contrast to the cerebellar peduncles, medulla and cord, there is much less of the état criblé. The demyelination is more complete and not attended by lacunar degeneration of the tissue. Figure 3 C illustrates lesions in the cerebellar white matter.

Areas may be seen similar to those in figure 2 *D*, i. e., small "holes" in the myelin caused by the heavily cuffed blood vessels. The intervening

10. King, L. S.: Arch. Path. **23**:338, 1937.

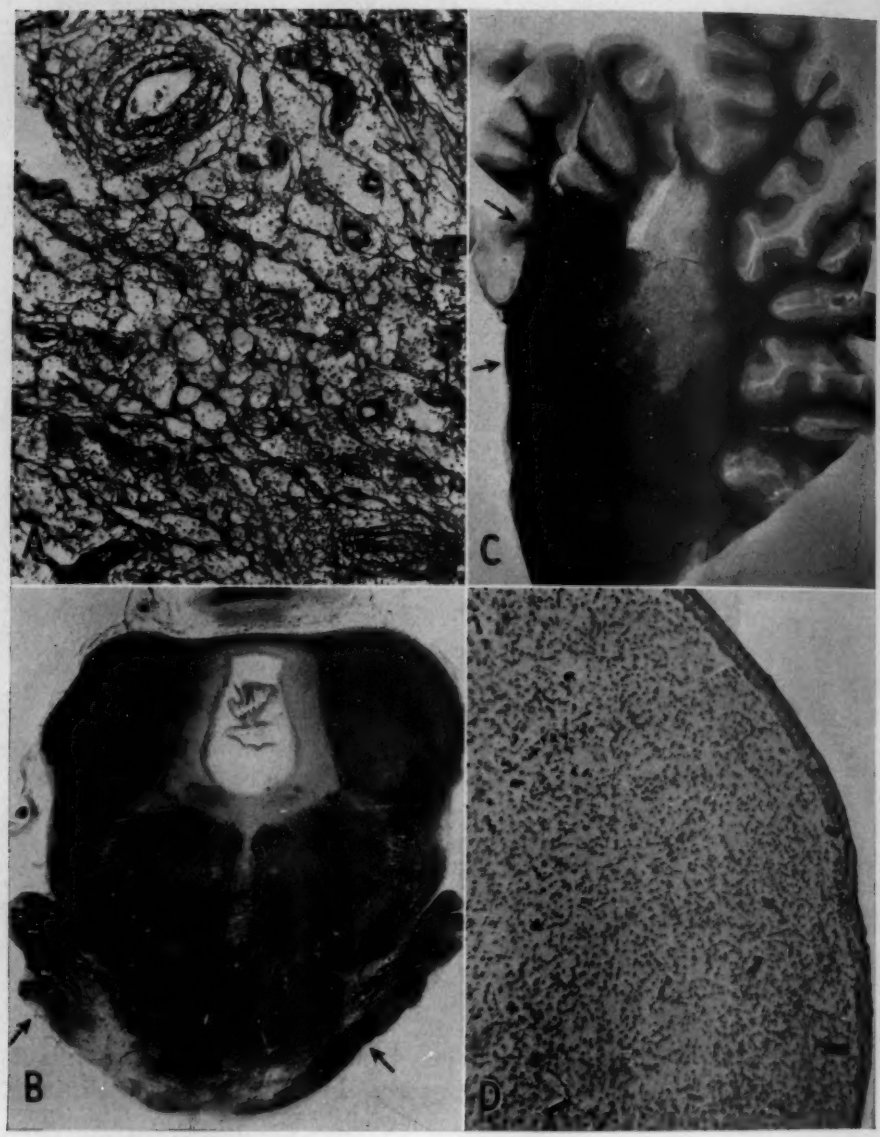


Fig. 3.—*A*, reticulin proliferation in a diseased area of the spinal cord. Silver carbonate impregnation for connective tissue; × 71.6. *B*, brain stem cut through the inferior colliculi and pons, showing demyelination in the pons. Arrows point to less obvious areas. Weil stain; × 3.6. *C*, demyelination in the cerebellum. Arrows point to early foci. Weil stain; × 4.25. *D*, inflammatory focus in the caudate nucleus, bordering the ventricle. Foci in the cerebral cortex are very similar. Hematoxylin-Van Gieson stain; × 30.3.

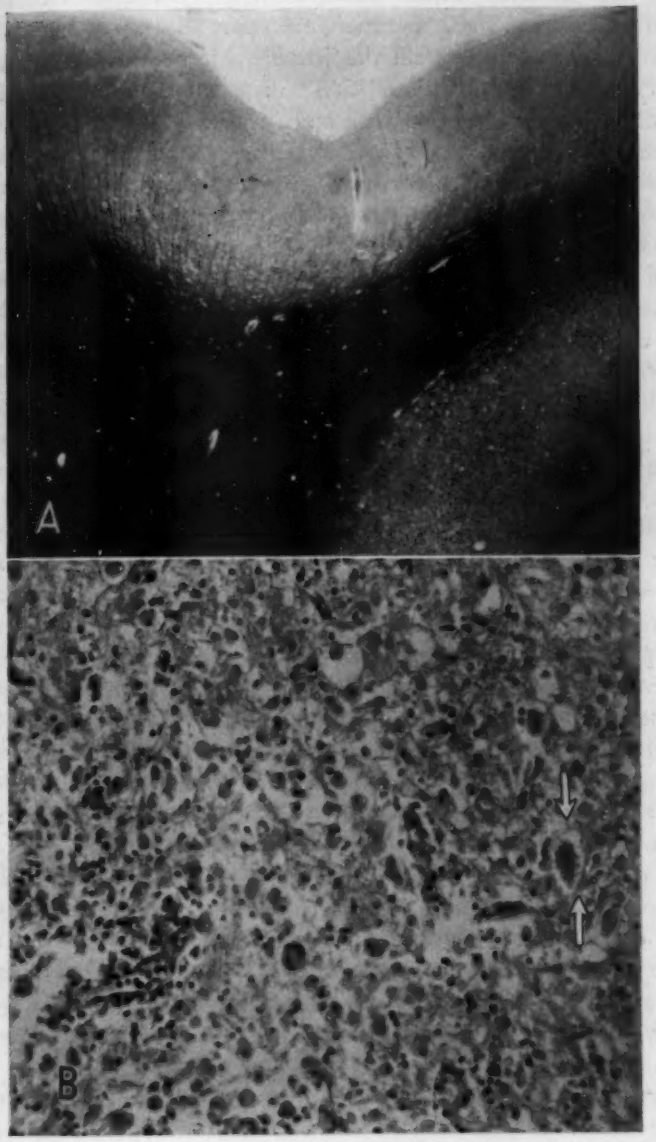


Fig. 4.—*A*, cerebral cortex, illustrating the loss of radial and tangential fibers in a cortical area of inflammation. Weil stain; × 18.4. *B*, tissue showing experimental canine distemper (from a preparation supplied by Dr. De Monbreun). The tissue is from the region of the dentate nucleus. The similarities to the picture described for the dog reported here are discussed in the text. The arrow points to a neuron with a prominent intranuclear inclusion. Hematoxylin-eosin stain; × 165.

myelin may at times appear lighter and less dense than in perfectly normal areas. (In the illustration, the partial involvement of the roof nuclei, naturally lighter than the white matter, should not cause confusion.) These "punched-out" areas usually appear, as in the spinal cord, at the margin or near much more severe damage.

The axis-cylinders may show moderate diminution in number and are nowhere as well preserved as they may be in some instances of multiple sclerosis. But the destructive action on the axis-cylinders is not so severe in the cerebellum as in the spinal cord. The usual degenerative alterations may occasionally be found.

There is considerably more gliosis, generally isomorphous in type, in the cerebellar lesions than in the spinal cord. Monster astrocytes are also present. The connective tissue proliferation and the reticulin nets, although present, are much less exuberant than in the spinal cord.

Occasionally in the cerebellar cortex there is an out-dropping of Purkinje cells, without vascular reaction. This change, fairly common in many pathologic conditions, is never severe.

Lesions such as have been described are also present in the white matter elsewhere in the brain—for example, in the optic chiasm and tracts and in the hippocampal commissure. But no damage has been detected in the corpus callosum or the centrum ovale, where Marinesco, Draganesco and Stroesco [3] described demyelination.

Scattered in the basal ganglions and cerebral cortex are numerous circumscribed lesions. The usual inflammatory changes are present around the blood vessels, and in the parenchyma there is some glial mobilization, as well as invasion in places by mononuclear cells. The nerve cells are in general excellently preserved, although there may be some increase in satellitosis. Figure 3 *D*, from the ventricular surface of the caudate nucleus, is typical of the lesions found both here and in the cerebral cortex. There is little true destruction of tissue, as found in the spinal cord. Appropriate stains, however, show loss of myelin. Figure 4 *A*, from the neocortex, shows loss of radial and tangential fibers at the site of inflammation. It will be observed that the underlying white matter is entirely normal. It is noteworthy that in these foci in the cerebral gray matter there is intense proliferation of fibrous astrocytes.

Careful search was made for intranuclear inclusions at all levels, but none was found.

COMMENT

This case is pathologically similar to those described by Perdrau and Pugh [1] and by Marinesco, Draganesco and Stroesco [3]; in all the condition may properly be called "disseminated encephalomyelitis with demyelination." The relation to distemper is not clear. Perdrau and

Pugh showed that this form of encephalomyelitis may occur long after, or even in the complete absence of, clinically recognizable distemper.

On the other hand, De Monbreun, studying distemper experimentally, produced and described lesions in dogs which appear to be similar to the demyelination described by others. Dr. De Monbreun sent me a preparation, a field from which is reproduced, with his permission, in figure 4 *B*. The field is from the region of the dentate nucleus of the cerebellum. The extensive damage of tissue, with numerous gitter cells, the inflammatory reaction around the blood vessels and the invasion of the parenchyma by inflammatory cells are clearly in evidence. (The neuron designated by the arrows shows a brilliantly acidophilic intranuclear inclusion, which cannot be well appreciated however in the photograph.) Such a lesion stained for myelin would show demyelination.

It is well established that in distemper as well as in other diseases the inflammatory changes in the nervous system are not of themselves sufficient to induce loss of myelin. The demyelination, according to Perdrau and Pugh,[1] "is not the result of the action of the specific virus of distemper on the central nervous system," but "the virus of distemper plays in this disease of the dog a similar role to that which an acute infection of varying etiology plays in the causation of certain demyelinating diseases of man."

From the work of previously cited authors the conclusion might be drawn that demyelinizing lesions may appear in the course of distemper, but also as an indirect, temporally removed consequence, as well as independently of this disease.

Certain features of the case reported here deserve comment. Different types of myelin loss may be seen. First, there is that which is attendant on practically complete destruction of tissue with inflammatory reaction (figs. 1 *B* and 2 *A*). Second, there is the strictly perivascular damage of myelin, appearing around heavily infiltrated blood vessels, where the distended perivascular sheaths have encroached on the parenchyma (figure 3 *A*). Third, there is the more diffuse and often incomplete loss of myelin, sometimes with état criblé. In the latter type there may be no inflammatory change in the parenchyma or varying degrees of change up to a very moderate intensity.

In considering the problem of demyelination it is necessary to distinguish sharply between destruction of tissue as a whole and more selective destruction of myelin. Various forms of encephalitis caused by viruses may produce a generally destructive process, in which all tissue elements are severely damaged. There is nothing selective about the process, and the myelin disappears only as a part of the general tissue disintegration. In animals this has been described especially in equine

encephalomyelitis.[11] There are many instances of myelitis and encephalomyelitis in man in which the observations are similar.

Opposed to the general damage of tissue, in which myelin suffers along with other tissue elements, there may occur in some diseases a selective destruction of myelin. In some instances of multiple sclerosis in which there is a sharp border of demyelination, the axis-cylinders within the lesion are essentially intact. Although gitter cells may be present in large numbers, there are generally no "inflammatory" cells. Such a picture, common in multiple sclerosis, was present to some extent in certain areas of the dog described here, especially in the cerebellar lesions. This selective attack on myelin is quite different from the general destruction of tissue found in many diseases proved to have viruses as their etiologic factors as well as in many of unknown cause.

A still different type of demyelination in the dog reported here is the état criblé, visible in figures 1 *A* and 2 *B*. This mode of reaction is quite different from multiple or diffuse sclerosis but is comparable to what is found in combined system disease. The problem of a dietary deficiency superimposed on the original disease process in this dog must remain open.

There are certain similarities to many of the reported cases of neuromyelitis optica, namely, marked involvement of the optic tract and of the spinal cord, with scattered lesions elsewhere. Very intense inflammation with widespread destruction of tissue is a feature of many instances of Devic's disease. It is only the latter cases that resemble the one reported here.

There has been much discussion in the literature regarding the possible nosologic unity of the different demyelinizing diseases. To me it seems clear that the disease described here, together with most of the disseminated encephalomyelitides with demyelination recorded in the literature, belongs in a totally different category from multiple sclerosis. Neuromyelitis optica belongs in or very near the former group. Diffuse sclerosis is not a disease entity, but certain of its subdivisions may be related to the first group, others to the second; still others may stand sui generis. These views are a matter of opinion only, as are other views expressed in the literature, and must remain so until further etiologic data are available.

SUMMARY

Disseminated encephalomyelitis with demyelination in a dog, of about three months' duration, is presented. There were intense inflammatory changes, with very severe involvement of the spinal cord, and scattered lesions elsewhere, including the optic chiasm and tracts. Different types of demyelination and the accompanying pathologic features are described.

11. King, L. S.: J. Exper. Med. **68**:677, 1938.

PRODUCTION OF XANTHOMA IN RABBITS

H. P. RUSCH, M.D.

C. A. BAUMANN, PH.D.
AND
B. E. KLINE, M.S.
MADISON, WIS.

It is generally believed that xanthoma is in some way associated with a disturbance in lipoid metabolism, but relatively little experimental work on the production of xanthoma has been reported. In rabbits fed cholesterol [1] lipoid deposits have been found in the sclera, the cornea, the ciliary body of the eye, the tympanic membrane and tendons. Anitschkow [2] produced typical xanthoma in rabbits by feeding cholesterol and at the same time traumatizing certain areas by injections of colloidin or turpentine. Since xanthoma nodules occurred at the injured sites, he concluded that two factors were necessary for their production—cholesteremia and trauma. Schaaf [3] produced xanthoma in 9 rabbits by feeding hydrous wool fat for periods up to seven hundred days. All of his animals were traumatized mechanically by a clamp on the neck and chemically by intradermal injections of lipoid mixtures. Furthermore, most of the animals suffered from a derangement of the liver due to action of roentgen rays, arsphenamine or para toluenediamine. Xanthoma appeared in the traumatized areas, but changes occurred also in other parts of the body, and the various complicating procedures failed to produce xanthoma in the absence of cholesteremia.

In connection with experiments on ultraviolet rays we have recently had occasion to feed cholesterol to rabbits for periods up to thirteen months and have observed xanthoma in the absence of trauma. In fact, xanthoma was produced whenever cholesterol was consumed for a long period, irrespective of other complicating factors.

From the Department of Physiology, University of Wisconsin Medical School.

This investigation was supported by the Jonathan Bowman Cancer Research Fund and by the Wisconsin Alumni Research Foundation.

1. Versé, M.: Virchows Arch. f. path. Anat. **250**:252, 1924. Berberich, J.: Klin. Wchnschr. **4**:2335, 1925. Kusnetzowsky, N.: Virchows Arch. f. path. Anat. **263**:205, 1927.

2. Anitschkow, N.: Arch. f. Dermat. u. Syph. **120**:627, 1914.

3. Schaaf, F.: Arch. f. Dermat. u. Syph. **175**:279, 1937.

EXPERIMENTAL PROCEDURE

Fourteen young adult female albino rabbits weighing from 1,680 to 2,500 Gm. were used. Eight of them were given a diet of Purina rabbit chow [3a] to which 0.23 per cent cholesterol and 3 per cent hydrogenated cotton seed oil (Crisco) had been added. Six controls were given only the Purina chow. The cholesterol was dissolved in hot Crisco, and this was poured over and thoroughly mixed with the pellets. The diet was consumed readily by the rabbits. Dried alfalfa was given twice weekly and water administered ad libitum. The animals were kept on shavings. When an animal died or was killed, sections were made of the liver, kidney, aorta and any pathologic tissue. Chemical analyses were made of the blood, liver, aorta and pathologic growths for cholesterol and fat. Six of the rabbits were irradiated sixty minutes daily with ultraviolet rays. After seven months Shope papilloma virus was injected into the ear vein or tattooed into the skin of the ear of each of 9 rabbits.

RESULTS

Except 2 of the controls, which died during the second and third months, all the animals gained in weight during the first months of the experiment and remained in good health. The average daily consumption of food per rabbit was 83 Gm., equivalent to 190 mg. of cholesterol. This is enough to cause cholesteremia and atherosclerosis but not enough to cause fatty liver.[4] After one month the cholesterol content of the blood of animals receiving cholesterol ranged from 3.58 to 12 mg. per cubic centimeter of whole blood, with an average of 6.17 mg. per cubic centimeter (table 1). In the control animals the average was 1.42 mg. per cubic centimeter. Analyses made after ten and twelve months indicated that the hypercholesteremia was maintained in all animals consuming cholesterol regardless of treatment with virus or ultraviolet rays.

There was no detectable difference in weight, food consumption or general well-being between the irradiated and the nonirradiated animals. The ears of the irradiated animals, however, were slightly red. Those given virus responded as indicated in table 1. The virus warts were

3a. The formula for Purina rabbit chow as furnished by the Ralston Purina Company, St. Louis, is as follows: wheat germ, soy bean oil meal, corn germ meal, alfalfa leaf meal, wheat middlings, ground oats, corn meal, blackstrap molasses, calcium carbonate and iodized salt. All these are mixed thoroughly and then pressed into pellets. The chemical analysis is: protein, 16.45 per cent; fat, 4.06 per cent; fiber, 7.93 per cent; ash, 3.75 per cent; nitrogen-free extract, 52.60 per cent.

4. Baumann, C. A., and Rusch, H. P.: Proc. Soc. Exper. Biol. & Med. **38**: 647, 1938.

usually confined to the ears, although some were scattered about other parts of the body.

Subcutaneous nodules developed in the feet of 7 of the 8 rabbits receiving cholesterol, regardless of other treatments. Most of the nodules were noted after ten months of cholesterol feeding, but in 1 animal nodules developed after six months. The nodules continued to increase in size until the death of the animals. They were either single or multiple, and some were confluent. The footpads on all the feet were

TABLE 1.—*Xanthoma Development in Rabbits*

Rabbit	Cholesterol in Diet	Ultraviolet Irradiation	Virus Treatment	Response to Virus	Cholesterol in Whole Blood, Mg. per Cc.	Xanthoma	Comment
31	+	+	None	.....	6.85	Large tumors on front and hindleg noted at 6th month	Died in 11 mo.
32	+	+	Intravenous	Warts on both ears	5.25	Numerous nodules on all feet	Died in 13 mo.
33	+	+	Intravenous	Few warts	5.30	Footpads enlarged	Died in 10 mo.
34	+	+	Tattooed	Large local papilloma	6.18	Numerous nodules on both feet	Died in 12 mo.
35	+	+	Intradermal	Few warts	3.58	.....	Killed in 9 mo.
36	+	+	Intravenous	Few small warts	5.03	Nodules on all feet	Killed for analysis at 12 mo.
38	..	+	Intravenous	Warts on ears	1.46	None	
39	..	+	Intravenous	Warts on ears	1.52	None	
41	..	+	None	.....	1.16	None	
42	..	+	None	.....	1.53	None	
43	+	..	Intravenous	Few warts	5.20	Numerous nodules on all feet	Killed for analysis at 12 mo.
44	+	..	Tattooed	Large local papilloma	12.00	Numerous nodules on all feet	Killed for analysis at 13 mo.

involved, becoming swollen, slightly tender and denuded. Occasional nodules were observed also at the knees and elbows. The size of the tumors ranged from that of a pea to 6 by 7 by 8 cm. The average size was about 1 by 2 by 3 cm. The largest tumor occurred on the left front foreleg of rabbit 31, near the elbow, and completely incapacitated the affected leg. The animal was extremely emaciated and died during the eleventh month.

The tumors were soft, rounded and white or pale yellow. In gross appearance they strongly resembled the nodules in patients with xanthoma (fig. 1). Most of them were tough and firm on being cut but

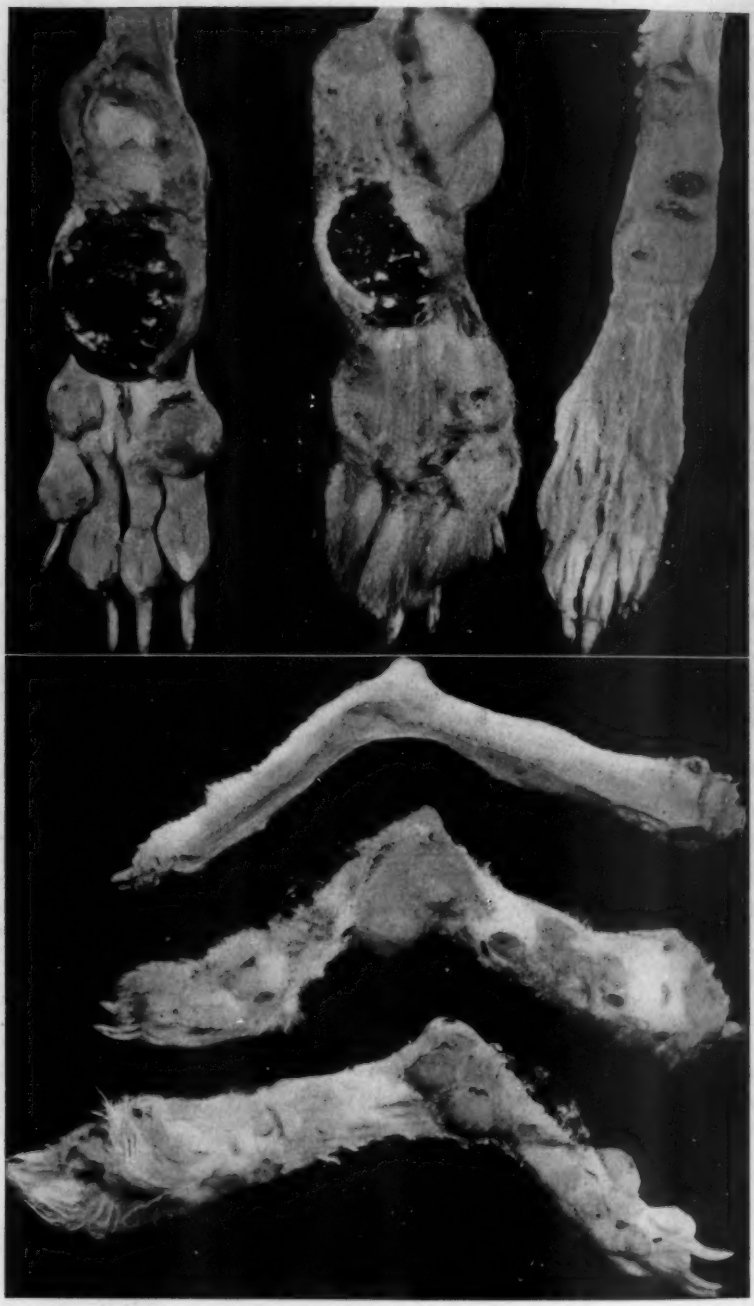


Fig. 1.—Xanthoma nodules of rabbits' feet. The foot without growths is that of a normal rabbit.

contained soft areas from which a white smooth substance the consistency of thick pus could be extruded with slight pressure. Others were less firm and contained small amounts of milky fluid. The nodules on the pads of the feet were firmer than those on the top and sides. Deposits of a yellowish white material were firmly incorporated in and around the tendon sheaths.

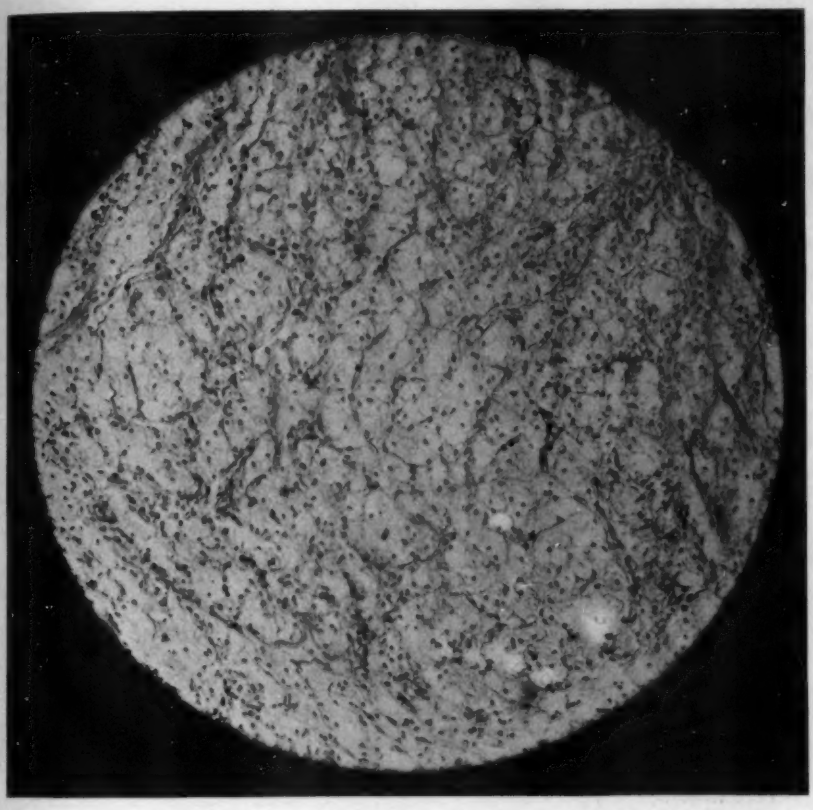


Fig. 2.—Photomicrograph of xanthoma nodule produced experimentally in a rabbit.

Histologically, the tumors were composed of masses of large foamy cells (fig. 2) loaded with lipoidal material. They appeared to be identical with the xanthoma nodules produced experimentally by Anitschkow and by Schaaf, and the foam cells, with the "xanthoma cells" or "cholesterin ester phagocytes" known in the human lesions.

Chemical analysis of 12 nodules taken from 3 rabbits indicated that fat constituted 16.6 per cent of the fresh weight or 46.4 per cent of the

dry weight of the tissue (table 2). The cholesterol content of the dried nodules ranged from 2.16 to 8.2 per cent, with an average of 4.57 per cent. Approximately one half of the cholesterol was in the free form.

Other changes accompanied the appearance of the nodules. The animals began to lose weight, and the hair became scanty on the back, neck, abdomen and legs. The underlying skin was wrinkled and had a yellowish cast. Some of the animals also lost hair about the eyelids, the skin of which was baggy and wrinkled. Numerous small white deposits were noted on the iris.

The subcutaneous fat of animals killed after twelve or thirteen months differed from normal fat in that it was more granular and less greasy. There was also marked atherosclerosis of the aorta. One case

TABLE 2.—*Fat and Cholesterol Content of Xanthoma Tissue*

Rabbit	Part Examined	Percentage of Fat, Fresh Basis	Percentage of Fat, Dry Basis	Percentage of Cholesterol, Dry Basis
32	Pad from front foot	19.8	41.8	3.94
	Pad from hind foot	21.6	48.4	5.45
	Nodule, hind foot	35.4	64.0	8.2
	Axillary lymph node	10.7	30.6	5.5
43	Popliteal lymph node	6.5	36.0	4.1
	Pad from front foot	11.6	46.5	2.6
	Pad from hind foot	14.0	50.1	4.2
44	Pad from front foot	18.4	50.8	5.11
	Pad from hind foot	14.8	47.5	4.49
	Pad from hind foot	10.5	42.5	5.41
	Pad from hind foot	28.6	68.0	3.72
	Popliteal lymph node	7.0	30.5	2 16
	Average	16.6	46.4	4.57

was noted in which this was so severe that all but one fourth of the normal opening was obliterated.

COMMENT

While our experiments were complicated by the fact that the rabbits were irradiated with ultraviolet rays or inoculated with virus in addition to being fed cholesterol in their diet, it is nevertheless evident that the cholesterol was the essential factor in the formation of xanthoma nodules. No effect due to ultraviolet rays could be demonstrated except a redness of the ears—which were not involved in the xanthomatous changes. In rabbits 31 to 36 (table 1), which received ultraviolet rays, nodules developed as large and as numerous as those in rabbits 43 and 44, which were not irradiated. In rabbits 38 to 42, which were irradiated but which received no cholesterol, xanthoma did not develop.

Similar evidence excludes the virus as a cause of xanthoma. Virus warts were confined largely to the ears; xanthoma nodules, to the feet.

There was, however, some overlapping of effects, for virus warts were sometimes found on the legs while xanthomatous skin changes were sometimes general. Nevertheless, there was no correlation between the response to the virus and the development of xanthoma. Xanthoma nodules developed in rabbits 34 and 44, which had large virus papillomas, exactly as in rabbits 33, 36 or 43, which had only a few transient virus warts (table 1). Rabbit 31, which received no virus, presented large xanthoma nodules. Rabbits 38 and 39 received virus but no cholesterol and did not show xanthoma nodules. Finally, the combination of the injection of virus and the ultraviolet irradiation failed to produce xanthoma.

The relationship between cholesteremia and human xanthomatosis has been pointed out frequently. Of 40 cases reported by 16 different authors, all but 10 were characterized by a definite increase in blood cholesterol.[5] In a case reported by Burns, blood fat, as well as blood cholesterol, was increased.[6] Arning and Lippmann[7] demonstrated an increase in neutral fat, lecithin and cholesterol in the case described by them. However, many patients with diabetes mellitus show hypercholesteremia but do not have xanthoma nodules. This probably means that a high level of blood cholesterol must be maintained for a long period before growths will appear.

In the rabbit, certainly, a long period is needed for nodules to accompany cholesteremia. The level of cholesterol feeding employed by us results in hypercholesteremia in two weeks and definite atherosclerosis within a month, yet xanthoma nodules did not appear for ten months.

Several investigators have considered hepatic insufficiency as the cause of xanthoma because the lesions are sometimes associated with jaundice. Chauffard and Laroche[8] found that in 8 patients with icterus there was also an increase in blood cholesterol, but the two conditions appeared to be relatively independent. Thus the highest cholesterol content was found in a patient with merely a subicteric tint. Their tests for the cholesterol content of skin removed from patients with jaundice also were inconclusive. The attempt to correlate xanthomatosis with pancreatic disease has produced no real evidence.

Little is known regarding the genesis of the disease. Harrison and Whitfield[5] stated that it is a process of infiltration dependent primarily on hypercholesteremia and secondarily on the duration of the blood condition, local trauma and the state of the local vascular supply. Pollitzer and Wile[9] claimed that the "xanthoma cells" arise from the

5. Harrison, G. A., and Whitfield, A.: Brit. J. Dermat. **35**:81, 1923.
6. Burns, F. S.: Arch. Dermat. & Syph. **2**:415, 1920.
7. Arning, E., and Lippmann, A.: Ztschr. f. klin. Med. **89**:107, 1920.
8. Chauffard, A., and Laroche, G.: Semaine méd. **30**:241, 1910.
9. Pollitzer, S., and Wile, U. J.: J. Cutan. Dis. **30**:235, 1912.

adventitial connective tissue cells of the smallest blood vessels of the papillary and subpapillary layers. As these cells become filled with cholesterol and increase in size and number they act as a stimulus to the connective tissue elements, fibroblasts and collagenous bundles, which surround the nests of xanthoma cells and ultimately predominate over the true xanthomatous elements. Basing his views on experiments with atherosclerosis in rabbits, Anitschkow [10] believes that the process attacks those parts which are composed of a dense framework of fibers, where the lymph circulates very slowly. The dense connective tissue, composed of various fiber systems with large surface area, acts as a barrier to the lymph flow with the result that the lipoids are arrested at these surfaces and undergo flocculent precipitation in some unknown manner. The lipoids are then phagocytosed by the lymphocytic and mononuclear wandering cells, which become the "xanthoma cells." Simultaneously fibrous proliferation occurs in this area. Our work as well as that of Anitschkow [11] demonstrates that xanthoma nodules occur principally in those parts which are composed of a dense framework of fibers. Because of the similarity here to the rabbit aorta, it appears that the production of "xanthoma cells" in other structures might occur in a similar manner.

SUMMARY

In rabbits receiving 0.23 per cent cholesterol in their diets for ten months xanthoma developed regardless of other treatments. Trauma is therefore not an essential prerequisite for the development of xanthoma.

10. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 298.

11. Anitschkow, N.: Charkowsky med. j. **22**:30, 1916.

SPONTANEOUS OPHTHALMIC MUTATION IN A LABORATORY MOUSE

FERDINAND L. P. KOCH, M.D.
NEW YORK
AND
JOHN W. GOWEN, PH.D.
AMES, IOWA

One of us (Dr. Gowen) in the course of his studies observed a spontaneous ocular mutation in one of his mice. The animal was the result of a cross between two long inbred lines, lines which up to that time had been continued by brother and sister matings. The fact that the animals of each mating were examined carefully both for their own defects and for those of their progeny makes it probable that this appearance of the character was its first within the colony.

Extracted progeny from this animal showed characteristic changes in animals of either sex. The strain has been continued by mating defectives. All animals from these matings have been defective. Animals of either sex taken from this strain are slow breeders. Mice showing ocular defects have appeared in the progeny of no other matings, although there are twelve other closely associated strains in which the syndrome could appear.

The ocular change shows wide variations in its manifestations. Animals at 10 days of age may show microphthalmic eyes. At the other extreme animals may appear normal till 3 months of age, when a central opacity may appear in either eye. The eyes within the same animal may show variation; one eye may be microphthalmic, while the other is normal in size but opaque. The eyes of the same animal may differ in time of onset of the disease. Consequently one may say that the character is quite variable in its expression. The eyes of several mice were examined both grossly and by means of the corneal biomicroscope prior to the killing of the animals. After enucleation, hematoxylin and eosin–stained serial sections of the paraffin-embedded specimens were studied microscopically. Several photomicrographs were taken of a typical section of each eye of one animal for this report.

The skull and extremities presented no manifest abnormalities, and the gross examination revealed both eyes to be normally situated. The right eye appeared to be normal in size, and the lids showed no abnormality; however, the cornea presented a large central opacity, and

From the Mayo Clinic (Dr. Koch) and the Department of Genetics, Iowa State College (Dr. Gowen).

the normal corneal reflex was lacking. The left eye was much smaller than normal, and the lids, particularly the margins, were occupied by approximating carrot-colored verrucous cornified masses. The cornea lacked luster and was very difficult to see through the small palpebral aperture. The left eye could not be examined with the corneal biomicroscope.

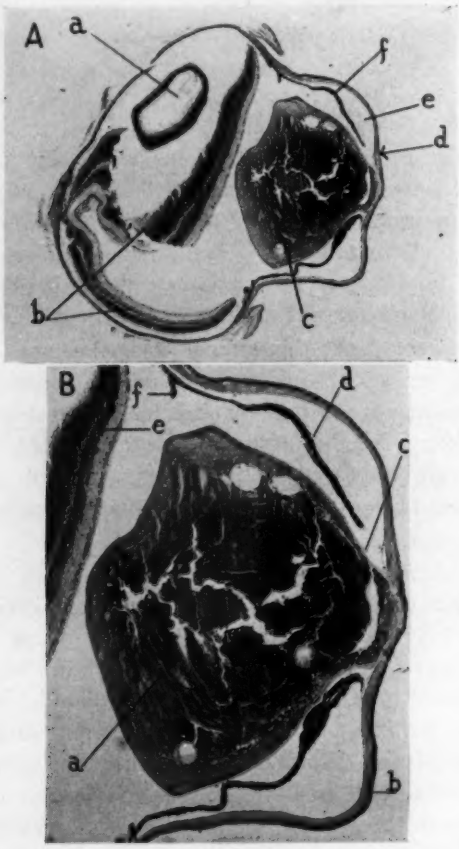


Fig. 1.—*A*, right eye; × 16: (*a*) artefactal invagination, (*b*) retina, (*c*) lens, (*d*) epithelium of the cornea, (*e*) anterior chamber and (*f*) iris. The relationship of the structures of the anterior segment is shown in this horizontal section. Posteriorly and above, the artefactal invagination of the sclera and choroid have resulted in a pushing downward and forward of the retina.

B, right eye; × 32: (*a*) lens, (*b*) epithelium of the cornea, (*c*) anterior capsule of the lens, (*d*) iris, (*e*) retina and (*f*) ciliary body. The rudimentary ciliary body, the heavily pigmented iris and the shallow anterior chamber are seen together with the intimate relationship of the lens and cornea.

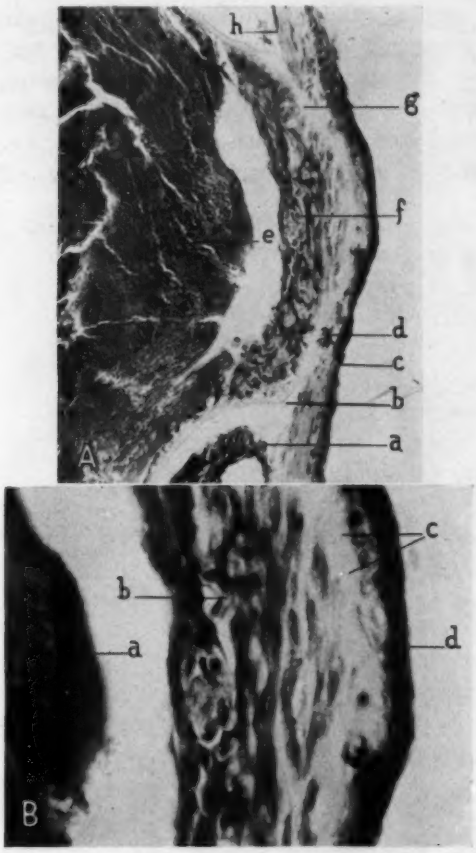


Fig. 2.—*A*, right eye; × 123: (*a*) iris, (*b*) anterior capsule of the lens, (*c*) epithelium of the cornea, (*d*) island of iris stroma and pigment cells, (*e*) lens, (*f*) anterior pyramidal cataract (proliferated subcapsular epithelial cells), (*g*) anterior capsule of the lens and (*h*) endothelium of the cornea. Small groups of pigmented iris cells are seen between the approximated posterior surface of the cornea and anterior surface of the lens. The anterior pyramidal cataract is seen as a bulging forward of the lens and consists of proliferated subcapsular epithelium. Below, the anterior surface of the iris meets the substantia propria and tends to continue forward and laterally (i. e., peripherally) along the substance itself.

B, right eye; × 290: (*a*) lens, (*b*) anterior pyramidal cataract (proliferated subcapsular epithelial cells), (*c*) anterior capsule of the lens and (*d*) epithelium of cornea. The homogeneous substance of the anterior capsule of the lens can be traced through the section as it lies in apposition to the thinned corneal substance. Behind the capsule lies the proliferated subcapsular epithelium. The corneal epithelium anteriorly is somewhat uneven in thickness. There is no evidence of infection.

The right cornea exhibited a large central opacification, which appeared to be connected intimately with the anterior surface of the lens. The periphery of the opaque region shaded off imperceptibly to clear, normal peripheral corneal tissue, behind which the anterior surface of a deeply pigmented structure, the iris, could be seen. Peripherally there was a very shallow anterior chamber, which was filled with clear

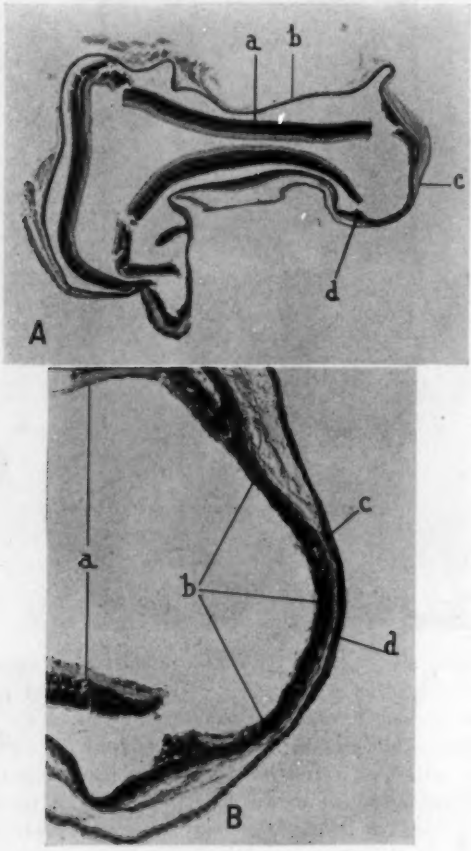


Fig. 3.—*A*, left eye; × 17: (*a*) retina, (*b*) sclera and choroid, (*c*) anterior surface of the cornea, the apex of which here is directed downward and somewhat to the right and (*d*) iris. Distortion has resulted from uneven fixation and paraffin impregnation. The anterior segment of the eye is seen to the right and is directed downward. The close relationship of the anterior structures is apparent.

B, left eye; × 77: (*a*) retina, (*b*) iris and pupillary membrane, (*c*) substantia propria of cornea and (*d*) epithelium of cornea. The eye is aphakic; there is no anterior chamber; the pupillary membrane is intact; the iris remains an embryonal structure and is adherent anteriorly to the cornea posteriorly. The ciliary body has not developed. There is no evidence of infection.

aqueous fluid. The leaf of pigmented tissue bowed forward to meet the posterior surface of the cornea at the junction of the latter with the anterior surface of the lens. The lens itself was rather opaque, and its deeper structures could not be observed in detail.

Microscopic examination of the serially cut sections revealed no evidence of previous intraocular infection. Typically, the posterior segment of the right eye exhibited no abnormality (figs. 1 and 2). Anteriorly, there was an anterior pyramidal cataract with compact and opaque anterior fibers but without actual reduplication of the capsule itself. There was rather marked proliferation of the subcapsular epithelial cells. The capsule itself was thickened and compressed against the posterior surface of a defective cornea. The lamina elastica posterior and most of the substantia propria in the immediate region of the contiguous compression were lacking. The lamina elastica anterior was thinned and at some points had ruptured, and the cornea exhibited rather marked epithelial proliferation. Occasional islands of iris stroma and iris pigment cells were seen between the anterior capsule of the lens and the disorganized corneal structure. The ciliary body was poorly developed, and the partially distorted and heavily pigmented iris appeared to persist centrally to remain united in front of the lens. This was thought to be a persistent pupillary membrane. Intact corneal endothelium could be traced down posteriorly to be lost within the encroaching iris at the point where the normal pupil probably should have existed. The pigmented iris cells did not spread appreciably on the posterior surface of the cornea as the former came in contact with the latter structure.

Microscopic examination of the left eye revealed apparently congenital microphthalmos, aphakia, opaque cornea and complete persistent pupillary membrane (fig. 3).

COMMENT

The defect in the right eye appears to have several components: (1) a defective cornea, (2) a persistent pupillary membrane, (3) a poorly developed anterior chamber and ciliary body and (4) an anterior pyramidal cataract. There is no microscopic evidence of prenatal or postnatal intraocular infection. Similar defects, careful search of the available literature shows, have not been reported in laboratory mice; however, it is possible that certain gross defects in albino mice which Pearson [1] described briefly might be an exception to this statement. Bagg and Little's [2] mouse variants, irregularly inheritable types, pos-

1. Pearson, E. S.: Nature, London **114**:433, 1924.
2. Bagg, H. J., and Little, C. C.: Am. J. Anat. **33**:119, 1924.

sibly induced by roentgen ray treatment of the mouse germ plasm, are other possible examples of defects showing similarity to ours. Rarely, profound malformations of the eyes are seen in instances of congenital cataract in man and, since similar anomalies have been bred pure in strains of rabbits, the defects unquestionably are inheritable (Friedenwald [3]). Mann [4] discussed the causes of congenital polar cataracts and of corneal developmental opacities in man and recalled the work of Collins.[5] The lack of information concerning the origin of congenital ocular defects in mice such as those reported here necessitates a search for an explanation by analogy among the known embryonic causes of similar defects in other vertebrates, particularly in man.

Supposititiously it appears that the defect in the right eye in this instance arose initially from a failure of the pupillary membrane to atrophy at the normal time during intrauterine development; i. e., the abnormality was an involvement, in its first phase, of the postendothelial (mesodermal) tissue, which is a structure that is very marked in rodents. This would account for the subsequent poor development of the anterior chamber and of the ciliary body with concomitant continuation of the approximation of the cornea and the growing lens through pressure. Thus, each of the two latter structures would have caused in one another by means of their enforced contiguity the development of cataractous changes and loss of normal corneal structural development, with subsequent occurrence of opacities in the lens and in the cornea, respectively.

The defects in the left eye—microphthalmos and aphakia—permit only speculation concerning their origin. There was seen no anterior chamber, and the cornea showed little or no development of the substantia propria and of the lamina elastica posterior; in addition, there was intimate union of the corneal tissue, over its entire posterior surface, with the iris stroma. The ciliary body, as such, was only rudimentary. It is probable that not only was there a failure of normal development of the mesodermal tissue in this eye but there was also a failure of the corneal ectoderm to react normally during early intrauterine development to produce a lens plate.

It is difficult to offer an unequivocal explanation for the appearance of spontaneous defects such as these on the basis of only a few microscopic observations in one generation, although grossly the defect regularly appears in all mice of successive generations within this strain. Further evidence will be presented with the progress of different phases of the investigation.

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3. Friedenwald, J. S.: The Pathology of the Eye, New York, The Macmillan Company, 1929.

4. Mann, I.: Developmental Abnormalities of the Eye, London, Cambridge University Press, 1937.

5. Collins, E. T.: Tr. Ophth. Soc. U. Kingdom **18**:124, 1898.

MEMBRANE FORMATION AT LIPOID-AQUEOUS INTERFACES IN TISSUES

II. A CORRELATION OF THE MORPHOLOGIC AND CHEMICAL ASPECTS

GEORGE M. HASS, M.D.*

BOSTON

Previous studies have shown that several liquid soluble unsaturated organic compounds which are relatively immiscible with aqueous tissue phases undergo gradual conversion into insoluble amorphous materials during a period in the subcutaneous tissues of guinea pigs.[1] Under appropriate conditions this conversion results in the formation of continuous membranes at the interface between the residual liquid compounds and the limiting tissues. These membranes resemble some naturally formed structures.[2] They offer an opportunity for investigating the gradual formation of complex interfacial films which can be visualized microscopically as they develop under biologic circumstances.

The study of these membranes has been conducted with four principal objectives in mind. The first objective was the distinction of a most elementary type of chemical structure, which was susceptible to the biologic mechanisms responsible for the transformation. As a solution of this part of the problem, data have been obtained which indicate that the simplest required chemical structures were long normal carbon chain acids or acyl groups with at least two ethylenic linkages in the chain.[1] A larger number of ethylenic linkages distinctly favored the transformation. These structural configurations alone were adequate for conversion but in the simplest case were not sufficient for the formation of long continuous interfacial membranes. The second objective, therefore, was the defining of an elementary chemical structure which was adequate not only for support of the biologic transformation but for support of membrane formation. In an approach to this problem, data have been obtained which indicate that some chemical alterations which accompany mild oxygenation of long normal chain highly unsaturated acids or acyl groups supply the added structural configuration which leads to the formation of continuous interfacial membranes.[2] Further efforts to

From the Department of Pathology, Harvard Medical School.

* Past Member Society of Fellows, Harvard University.

1. Hass, G. M.: Arch. Path. **26**:1196, 1938.
2. Hass, G. M.: Arch. Path. **27**:15, 1939.

define the general nature of these alterations have been made, and the results will be reported on subsequent pages.

The preceding data have indicated general lines of procedure for approaching the third objective, namely, a determination of the fundamental structure of these interfacial membranes, as they are constructed in the biologic environment under investigation. Data pertaining to this aspect of the problem will be presented in this report.

The final step in this investigation follows in natural sequence and will encompass all attempts to formulate such chemical and morphologic rules of behavior of compounds in this semibiologic system as might be applied to natural systems under more nearly physiologic circumstances.

Among several possible approaches to this aspect of the problem, the following have been made, and the experimental results will be recorded here. First, extracts of several mammalian tissues have been prepared for purposes of demonstrating whether materials amenable to the transformation are present in normal tissues. Second, two representative classes of natural esters, cholesterol esters and lecithin, which often have highly unsaturated acyl groups, were added to the list of compounds under investigation. Third, experiments were devised to determine whether the transformation is possible in bodily sites other than the one previously investigated. Fourth, attention was directed to the possible participation of protoplasm in the structure of the membranes.

METHODS

Experiments designed to compare the transformation of acid-free cod liver oil in the subcutaneous tissues of guinea pigs with the behavior of the oil in other tissues were done as follows: Acid-free cod liver oil (0.2 cc.) was injected directly into the spleen, liver, kidney, pancreas and testis. Intravascular injections were made by way of the ventricles of the heart and the spleen. The intraventricular injections were always followed by death of the animal within a few hours and consequently were unsatisfactory. Intrasplenic injections were consistently satisfactory and yielded an opportunity to study the transformations of the oil in the spleen, liver and lungs, the oil having been transported from the spleen to the liver and lungs by the vascular route. The experiments were terminated after intervals of two or three weeks. The tissues were fixed in solution of formaldehyde U. S. P. (10 per cent), and representative blocks were embedded in paraffin. Hematoxylin and eosin, Weigert's stain for elastic tissue and Ziehl-Neelsen carbolfuchsin stain were used routinely on paraffin sections.

Preparations of materials from mammalian tissues for the purpose of demonstrating the presence of compounds amenable to the transformations in question were made as follows: Fresh guinea pig brains and livers and human brain tissue were used. These tissues were macerated in separate containers and then extracted with alcohol. After several days the residue was separated by filtration and extracted with peroxide-free ether for forty-eight hours. The ethereal extract in each instance was concentrated until the solution was highly viscous. The subcutaneous injections and the preparation of tissue for microscopic study were made in the usual manner.

Blood was obtained from 4 different persons. Immediately after 25 to 50 cc. of blood was drawn by venous puncture, it was placed in acetone. After several hours the acetone was removed by filtration, and the residue was extracted for at least twenty-four hours with ether. The acetone and ether extracts were combined and evacuated to dryness. The dried material was taken up into a small amount of purified petroleum benzine and injected in solution. The injections were made subcutaneously, and the tissues were prepared for study by methods previously described.

As cholesterol esters of unsaturated long chain organic acids which can be conveniently purified are solid at 37.5 C., liquid cholesterol esters were prepared as follows: Fifteen grams of highly unsaturated acids of cod liver oil obtained by methods previously described was mixed with 10 Gm. of cholesterol (melting point 145 to 146 C.). The solution was agitated gently in the presence of a slow stream of dry hydrochloric acid at 35 C. for ten minutes. Free hydrochloric acid was evacuated, and the mixture was then heated in an atmosphere of carbon dioxide for one hour. The products were taken up in ether. The ethereal solution was washed free of acids with dilute aqueous potassium hydroxide. The ether was distilled off. The products were fractionally crystallized from alcohol over a period of several days, a small amount of water being added to the alcohol after the removal of each crop of crystals. Finally, a residue of about 1.5 Gm. of liquid esters was obtained. This mixed product formed liquid crystals. It began to solidify at about 30 C. and was almost entirely solid at 15 C. Cholesterol and liquid fatty acids in amounts in accord with approximate calculations were obtained by hydrolysis of a small amount of the material. This liquid product was injected subcutaneously in doses of 0.2 cc. After intervals of two or three weeks, the tissues were prepared as usual for study.

Lecithin was purified and administered in the following manner: Ten grams of "egg lecithin" (Merck) was dissolved in alcohol. An excess of cadmium chloride was added to the solution. The precipitate was thoroughly refluxed in ether. The ether-insoluble material was dissolved in a mixture of boiling ethyl acetate and alcohol. On cooling, the cadmium chloride–lecithin addition compounds precipitated. The precipitate was removed by centrifuging and subsequently dissolved in chloroform. The lecithin was set free from the cadmium chloride by addition of ammonia in alcohol to the chloroform solution. The precipitate was removed by centrifuging. After evaporation of the chloroform, the lecithin was dissolved in ether and precipitated quantitatively with acetone. The lecithin so obtained was almost pure white, soft, plastic and highly hygroscopic. After the lecithin had been dried in high vacuum, saturated solutions of lecithin in high-boiling paraffin oil, olive oil and butyl oleate were prepared. Two-tenths cubic centimeter of each saturated solution was injected subcutaneously into guinea pigs. After intervals of two or three weeks the tissues were fixed and stained in the routine manner.

The study of the relation of tissue proteins to the lipoidal membranes was conducted by microscopic examination of a considerable amount of material available. In addition to the stains mentioned previously, Mallory's aniline blue–orange G stain for connective tissue, osmic acid stains of fresh tissues and the Swank-Davenport modification of the Marchi stain for degenerating myelin were used.

The influence of several common oxidizing and reducing agents was studied in the following manner: The reducing agents employed in vivo were hydroquinone

and pyrogallol. Each of these compounds was added (2 to 5 mg. per gram) to separate samples of acid-free cod liver oil and to oxygenated methyl esters of unsaturated acids derived from cod liver oil. The oil and mixed esters, each containing a reducing agent in solution, were injected subcutaneously. Appropriate control experiments were done. The animals were killed at the end of two or three weeks. The tissues were treated in the routine manner.

The study of the action of oxidative catalysts in vivo was made as follows: Cod liver oil (acid-free), methyl esters of unsaturated acids derived from cod liver oil and oxygenated methyl esters of unsaturated cod liver oil acids were employed. Various finely pulverized ferric salts (ferric chloride, ferric stearate and ferric oleate) and ferrous chloride were added to samples of each material, and after centrifuging, the oil and esters containing iron salts in solution and colloidal suspension were injected subcutaneously. The tissues at the end of ten days, two weeks or three weeks were treated in the routine manner. An additional stain, the prussian blue stain for iron, on sections cut from paraffin was used in this series of experiments.

In previous studies several comments have been made with regard to ferric chloride–oxygen treatment of the liquid unsaturated esters in vitro and concerning permanganate oxidation of fixed and mounted preparations. An extension of the study of the action of oxidizing agents in vitro was conducted by direct fixation of fresh tissues in 1 per cent aqueous osmium tetroxide (perosmic acid) and by employing the Swank-Davenport modification of the Marchi method on tissues fixed in solution of formaldehyde. Acid-free cod liver oil, oxygenated methyl esters of unsaturated acids of cod liver oil and linseed oil plus 20 per cent unsaturated acids of linseed oil were injected into guinea pigs subcutaneously. After intervals of two or three weeks, selected blocks of tissue at the sites of injection were prepared for study by direct fixation in 1 per cent perosmic acid for twenty-four hours, followed by rapid dehydration and paraffin embedding. Adjacent blocks of tissue were fixed in solution of formaldehyde and prepared according to the Swank-Davenport method for staining degenerating myelin.[3]

As unsaturated methyl esters of cod liver oil acids do not form continuous membranes in the tissues unless they have been subjected previously to oxygenation, a preliminary investigation of the oxygenated materials was conducted, with the idea of demonstrating the creation of polar groups at points of unsaturation as the result of partial oxidation. For this purpose, methyl esters of unsaturated acids of cod liver oil (50 Gm.) were oxygenated by bubbling air through them for seven days. The products assumed a yellowish tinge and became increasingly viscous, some materials either settling to the bottom or adhering to the glass wall of the oxygenating apparatus. All dissolved easily in ether. The ethereal solution was washed with aqueous potassum hydroxide, and after the ether was distilled off, the quantity was divided into two equal parts. One was treated in the usual way with phenylhydrazine and acetic acid, chloroform being used as the solvent. The other was treated with benzoyl chloride, pyridine being used as the required base and chloroform as the solvent. There were appropriate controls. In each instance, after concentration of the reacting mixtures and addition of benzene, a crystalline precipitate was obtained. The compounds which were regenerated by hydrolysis of the hydrazones and benzoyl derivatives were brownish liquids. They were unstable and became so altered during the manipulations that they were unfit for further dependable study.

3. Swank, R. L., and Davenport, H. A.: Stain Technol. **10**:87, 1935.

Recourse, therefore, was made to an application of methods for demonstrating carbonyl and hydroxyl groups of compounds in solution to this problem, in which I had to deal with insoluble materials in paraffin sections of tissues mounted on glass slides. Phenylhydrazine, phenylhydrazine hydrochloride and hydroxylamine hydrochloride were selected as reagents for the carbonyl group. Benzoyl chloride and acetic anhydride were chosen as reagents for possible condensation with hydroxyl groups. Chloroform or alcohol was used as a solvent for the reagents. After immersion of thin sections of tissue mounted on glass slides in the solvents, the specific reagents and adjunctive reagents were added. The methods were the same as those generally in use in work with materials in solution and will not be discussed fully here. The preparations were allowed to stand at room temperature in the dark for periods varying from three to twenty-four hours. The slides with tissues mounted on them were then washed in alcohol and water. The tissues were subsequently stained by Weigert's resorcinol-fuchsin method. The membranes were studied microscopically and the intensity of election of the resorcinol-fuchsin stain graded directly by color vision.

Two types of membranes were investigated by these methods, one arising by transformations of cod liver oil and the other by conversion of oxygenated methyl esters of unsaturated acids of cod liver oil. Eleven experiments have been completed on sections of tissue mounted in series. The results include data on the influence on the intensity of election of Weigert's resorcinol-fuchsin stain by membranes treated as follows: (1) by a "keto-fixing" reagent, (2) by a "hydroxyl-fixing" reagent, (3) by a "keto-fixing" reagent followed by a "hydroxyl-fixing" reagent and (4) by solvents and other adjunctive reagents necessary for making the determinations.

EXPERIMENTAL RESULTS

Cod liver oil (acid-free) after direct injection into the spleen, liver, pancreas, kidney and testis was partially transformed into the characteristic insoluble semisolid materials. If the cod liver oil was introduced intravascularly by way of the splenic sinuses, the same general types of alterations occurred and were noted in the splenic pulp, the liver and the lungs. In the several sites of localization there were minor differences in the rate and extent of transformation of the oil. The opportunities for the persistence of long continuous lipoid-aqueous interfaces in the lungs, liver, kidney and spleen were of course greatly restricted. Perfectly formed membranes entirely similar to those described previously in the subcutaneous tissues were routinely found at extended lipoid-aqueous interfaces in the pancreas and testes. In no instance were the altered lipoids demonstrable in epithelial or mesenchymal cells other than those which are generally believed to have phagocytic capacities. In general, extracellular transformed products were easily demonstrable, and all studies indicated that intracellular conversion also occurred in the same way as in the subcutaneous tissues.

The experiments with extracts of human brain and whole blood as well as those with extracts of guinea pig brain and liver showed that in the ether-soluble and acetone-ether-soluble fractions as prepared there were materials which underwent alterations analogous to those under

discussion. The altered products were not abundant and were always present in the form of small globules and granules. No long continuous lipoid-aqueous interfaces were seen in the tissues, so that no conclusions could be drawn with respect to the presence of compounds suitable for transformation into membranes at such boundaries. The principal part of the insoluble materials which elected Weigert and Ziehl-Neelsen stains was in the cytoplasm of macrophages and multinucleated giant cells.

At the sites of injection of liquid cholesterol esters of unsaturated acids of cod liver oil there was a severe inflammatory reaction with extensive necrosis of tissues. Clefts in which cholesterol crystals were embedded were common. These clefts were often marginally limited by multinucleated giant cells. These details indicated that partial hydrolysis of the esters had occurred during their residence in the tissues. There was a fairly large amount of insoluble material derived either by transformations of the esters or their products of hydrolysis. This material was acid fast and took Weigert's resorcinol-fuchsin stain. It was principally in the form of small globules and granules, although occasionally long continuous delicate interfacial membranes were encountered. These resembled those which have been described previously around residual deposits of oxygenated methyl esters of unsaturated acids of cod liver oil.

No insoluble material which elected Weigert's stain or exhibited acid-fast properties by the Ziehl-Neelsen method was found at the sites of injection of purified egg lecithin.

The protoplasmic components which were most frequently present on the aqueous aspect of the lipoid-aqueous interface were collagen, fibrin and cytoplasm. The relation of these elements to the membranes may be described as follows:

Homogeneous or fibrillar collagen was occasionally the only distinguishable morphologic element on the aqueous aspect of the interface (fig. 1 *A*). This was not only preformed collagen but also that which had been deposited during the period of membrane formation. A line of demarcation between the altered lipoids and the limiting collagen could not always be distinguished. In other words, there was a narrow interfacial region where fusion of liquid or fibrillar collagen and altered lipoids seemed to occur. In such areas the membranes were sharply and deeply stained on their lipoidal aspect. This sharp staining, illustrated best by Verhoeff's stain for elastic tissue, gradually became less intense as the interface was approached and merged into the counterstain characteristic for the limiting collagen. These relations were also clearly shown with Mallory's aniline blue stain for connective tissue. Normal collagen was stained blue as usual. The principal part of the membrane was stained yellow. At points of fusion of the yellow altered lipoidal components of the membranes with the underlying blue collagen,

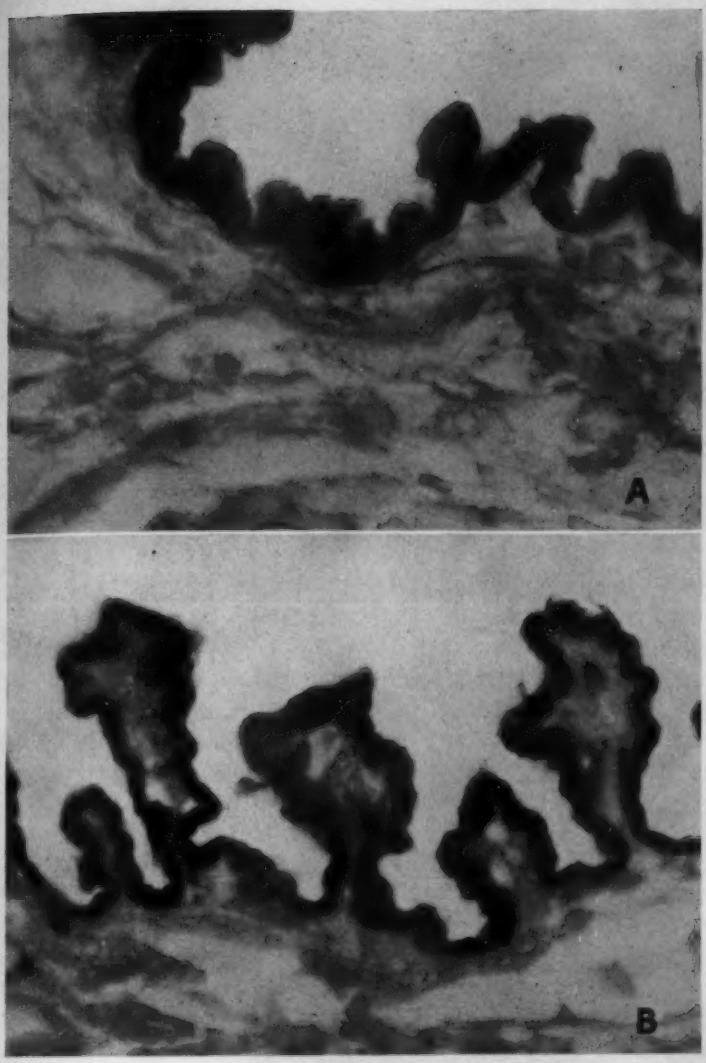


Fig. 1.—*A*, section showing transformation of oxygenated methyl esters of highly unsaturated acids of cod liver oil plus a ferric chloride catalyst in subcutaneous tissues for two weeks; formaldehyde fixation; Weigert stain for elastic tissue. The clear space represents the site of the soluble residual components of the ester globule. The wavy black structure (about 8 to 10 microns thick) is the membrane which has formed at the interface between the ester and the adjacent aqueous protoplasm. Note the limiting fibrillar collagen at the base of the membrane. The delicate black fibrils which extend from the region of the interface in the plane of the collagenous fibrils may be preformed elastic fibrils, but there are no readily applicable methods which may be depended on to distinguish them as wholly independent of the interfacial membrane. *B*, section showing alterations of oxygenated methyl esters of highly unsaturated acids of cod liver oil in tissues for four weeks: formaldehyde fixation; Weigert stain for elastic tissue. The clear space is the site of residual esters which have been dissolved by the treatment of the tissues with alcohol and chloroform. This photomicrograph illustrates the highly convoluted continuous membrane which has formed at the ester-protoplasmic boundary. The underlying collagen, which by the method used stains gray rather than black, which is characteristic for the membrane, is closely applied to the base of the membrane and is intimately fused with it, extending into the numerous folds so as frequently to be attenuated to the point of invisibility.

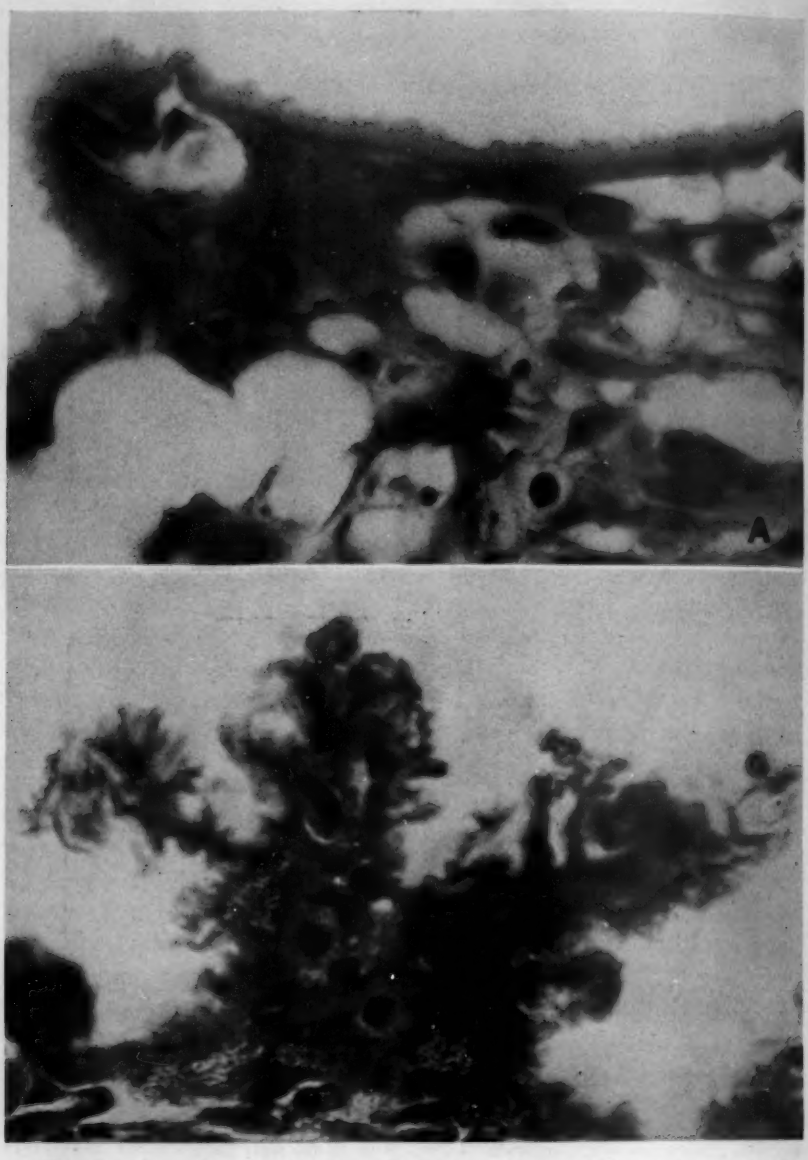


Fig. 2.—*A*, section showing the effect of acid-free cod liver oil in the subcutaneous tissues for two weeks; formaldehyde fixation; Verhoeff's stain for elastic tissue. The clear space above the curved margin represents the site of the soluble components of the oil globule. The marginal grayish black zone (about 5 or 6 microns in breadth) is the interfacial membrane. This is sharply limited basally by a continuous layer of cytoplasm. Note the condensation at the line of contact between the cytoplasm and the interfacial membrane. *B*, reaction showing effect of acid-free cod liver oil in the tissues for two weeks; formaldehyde fixation; Verhoeff's stain for elastic tissue. The clear space is the site of residual soluble oil. The highly complex margin shown in this photograph was initially the curvilinear margin of a spherical globule of oil. This illustration shows, as well as can be shown within the limits of photographic reproduction, the arborescence of limiting protoplasm at the interface. Each delicate protoplasmic projection is covered by a thin layer of the lightly stained interfacial membrane.

a narrow zone of greenish coloration was often noted. This region, as a rule, remained optically homogeneous, and only rarely were collagenous fibrils demonstrated as a basal component of the membranes. The fibrils were principally oriented as limiting structures on the aqueous aspect of the interface. As such they were often firmly bound to the membranes and remained adherent to them where the membranes had been mechanically dislocated from their site of formation. Therefore, in some areas the following layers were distinguished: first, the peripheral zone composed of altered lipoids; second, a narrow zone of altered lipoids fused with collagen; third, a layer of fibrillar or liquid collagen, and fourth, a limiting margin of mesenchymal cells.

This arrangement was also detectable in perosmic acid and Swank-Davenport preparations. In these sections thin films of the artificially introduced compounds were occasionally present between delicate collagenous fibrils at the boundary. These were visualized in two dimensions as delicate layers of lipoid alternating with protein (fig. 3*A*).

The participation of collagen in the structure of membranes at the interface was always greatly limited. It seemed that surface forces and simple restricted diffusion would account for most of the microscopic picture, although there were no reasons for denying the probability of formation of lipoprotein compounds by chemical interaction.

The preceding account of the participation of collagen in the structure of the membranes was in general applicable when fibrin was concentrated on the aqueous aspect of the interface.

The interpretation of the histologic picture when cell margins formed a limiting boundary at the interface was difficult. The margins of cells, especially elongated fibroblasts and multinucleated giant cells, were usually sharp and well defined (fig. 2*A*). In other instances the cytoplasm was apparently so fused with the altered lipoidal compounds that no cell membrane was distinguishable (fig. 3*B*). This apparent peripheral coalescence of protoplasm with the altered lipoids at the interface was demonstrable with all types of stains used. Not infrequently a line of cleavage was present, so that the marginal protoplasm was detached from the body of the cell and remained bound to the lipoidal component of the membrane. All data favored the conclusion that the lines of cleavage were artificially produced by trauma incidental to the preparation of tissues for study. Nevertheless, there was no hesitancy in concluding that peripheral cell protoplasm was occasionally so modified by its contact and fusion with altered lipoids that it formed, from the histologic point of view, a basal component of the membrane.

The binding of collagen, fibrin and cell protoplasm to the altered lipoids of which the membranes were principally composed resulted in several interesting architectural arrangements of lipoid and protein. Over portions of the membrane boundary the proteins formed a

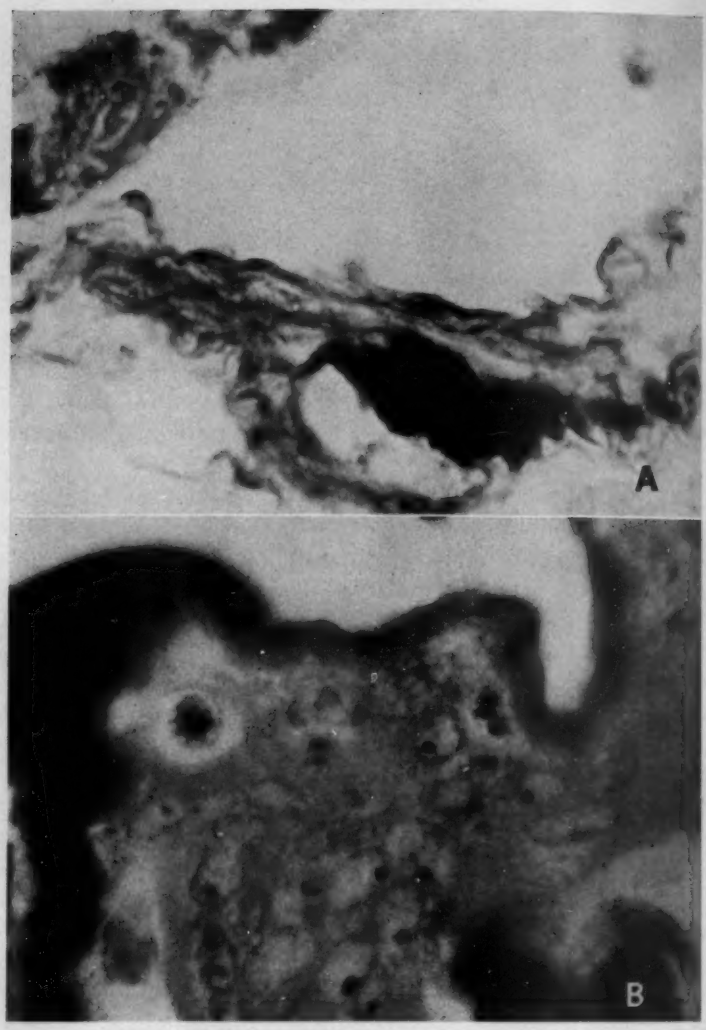


Fig. 3.—*A*, section showing the effect of methyl esters of unsaturated acids of cod liver oil in the subcutaneous tissues for two weeks; direct fixation and staining with 1 per cent aqueous osmium tetroxide. This field shows clear areas from which residual esters have been dissolved during preparation of the tissues and black areas of residual esters which have become insoluble in the dehydrating agents and which have retained their capacity to reduce osmium tetroxide. Note the thin black films of lipoids which extend between delicate collagenous fibrils for variable distances from the three major ester-protoplasmic interfaces shown in the photograph. *B*, section showing the effect of oxygenated methyl esters of unsaturated acids of cod liver oil in subcutaneous tissues for three weeks; formaldehyde fixation; Swank–Davenport modification of the Marchi stain. The clear space at the upper margin represents the site of residual esters which remained soluble in the dehydrating agents despite the treatment with the perchlorate-perosmic acid solution. Adjacent to this clear area is a discontinuous black zone of esters which reduced perosmic acid and remained insoluble in the dehydrating agents. Beneath this interrupted layer there is a pale gray interfacial membrane, which has largely lost its capacity to reduce perosmic acid. This section of membrane is representative of certain segments which cannot be clearly demarcated from the cytoplasm of adjacent cells (in this instance a multinucleated giant cell).

tangential marginal component which was either homogeneous or fibrillar (fig. 1*A*). Over other portions of the boundary the protein formed a tangential component composed of fibrils alternating with thin films of lipoid (fig. 3*A*). Over still other parts the membranes were often convoluted and wavelike on cross section (fig. 1*B*). The lengths and amplitude of the waves varied greatly. Where wavelengths were short and amplitudes great, the proteins at the boundary so conformed that in the extreme case they were disposed in a direction transverse to the general plane of the membrane and tangential to the plane of the front of the waves. At times the protein so included was attenuated to the point of invisibility (fig. 2*A*). Whether it disintegrated or became fused wholly in the membrane was a question not solved by the methods employed. The arrangement which has been described occurred irrespective of the protein implicated.

Hydroquinone or pyrogallol if dissolved in compounds prior to injection into the subcutaneous tissues did not alter either the rate or degree of transformation of the compounds in vivo. Neither did either material interfere with the mechanisms responsible for the formation of membranes.

The presence of ferric or ferrous compounds in the materials during their residence in the subcutaneous tissues resulted in pronounced acceleration of the transformation of the various unsaturated compounds in vivo. This was independent of the nature of the iron-bearing compound used. In general, the extent of alteration in the presence of iron salts at the end of ten days or two weeks compared favorably with the degree of conversion of control materials at the end of three or four weeks. The morphologic end result of the transformation in each instance was essentially the same.

The distribution of iron as demonstrated by the prussian blue stain on paraffin sections was variable. The relation of iron to the intracellular transformed compounds and to the extracellular long continuous membranes was of interest. In macrophages and multinucleated giant cells the pattern of distribution of iron demonstrated with prussian blue often coincided with that which had been observed in occasional zones of reaction previously studied, in which no iron had been added to the compounds employed. In this instance, it was assumed that the iron was derived principally from disintegrating hemoglobin.

The continuous membranes showed in many areas a type distribution of iron which was sufficiently frequent to warrant comment. In the region of the junction of the lipoidal aspect of the membrane with the limiting cell margins a row of iron granules was seen. A similar planar distribution of iron was occasionally demonstrated on the opposite margin of the membranes. When both distributions of iron were present

in the same segment of membrane, the altered lipoid appeared to be encased in a more or less continuously distributed sheath of iron. In addition to these distinct linear arrangements, a delicate diffuse blue staining of the membranes was occasionally noted. This was so prominent in several sections of membrane that it was detectable by low power microscopic examination. Iron otherwise was distributed at random, although in the cytoplasm of phagocytic cells it was often in association with ingested lipoidal granules.

The action on the altered lipoids of aqueous osmium tetroxide (1 per cent) or aqueous osmium tetroxide in solution with formaldehyde, potassium perchlorate and acetic acid (as required in Swank and Davenport's modification of the Marchi method) may be described as follows: Although there were minor differences according to the method used, these have not been carefully worked out. The general results may be considered as similar for my purposes at this time. The compounds which were used were of such nature initially as to effect a reduction of osmium tetroxide by either method. In the course of biologic conversion of the compounds they gradually lost their initial capacity for reducing perosmic acid. This was more satisfactorily demonstrated by the Swank-Davenport method. By either method a part of the residual compounds which lay adjacent to the membranes was converted so as to be resistant to the action of the solvents employed in preparation of the tissues. Thus these preparations showed, first, the internal materials which were stained black by the reduction compounds of perosmic acid and, second, the marginal materials which had either partially or completely lost this reducing power (fig. 3*B*). These sections of membranes, filaments, globules and granules lightly retained the fuchsin counterstain.

The methyl esters of unsaturated acids of cod liver oil after seven days' oxygenation in vitro contained liquid soluble compounds which formed crystalline derivatives with both phenylhydrazine and benzoyl chloride. The yield of crystalline derivatives was small in each instance. On hydrolysis of these compounds a viscous dark liquid which was unfit for further experimentation was obtained. Although these observations indicated that keto and hydroxyl groups had been created by oxygenation of the unsaturated compounds, the small yields and instability of the regenerated products were strong arguments against this approach to the problem of chemical groups operating in the biologic formation of membranes.

The results of chemical tests which were made on the lipoids in thin sections of tissue mounted on slides were as follows:

A treatment with phenylhydrazine, phenylhydrazine hydrochloride or hydroxylamine hydrochloride produced such alterations as to bring

about an inhibition of the election by the membranes of the resorcinol-fuchsin stain. In some instances the inhibition was practically complete. Unfortunately, phenylhydrazine and its hydrochloride gave lightly colored yellowish solutions under the experimental conditions. The membranes acquired a similar color during the treatment, and this interfered with the interpretation of the intensity of staining by Weigert's method. If this yellowish coloration was a consequence of direct dyeing, the altered lipoids were the only components in the tissues which were so stained. Hydroxylamine hydrochloride did not give colored solutions in these experiments, and the results from its use served as a fairly satisfactory check on the interpretation of results when sections were treated with phenylhydrazine or phenylhydrazine hydrochloride.

The results of attempts to interfere with resorcinol-fuchsin staining of altered lipoids by a previous treatment with benzoyl chloride or acetic anhydride were not so satisfactory. There was constant demonstrable inhibition of election of the stain by lipoids subjected to the action of these reagents, but it was invariably slight.

The treatment first with a "keto-fixing" reagent and second with a "hydroxyl-fixing" reagent usually but not always diminished the inhibition obtained by use of the "keto-fixing" reagent alone.

Many controls were tested simultaneously. The only accessory reagent or solvent that interfered with the resorcinol-fuchsin reaction was sulfuric acid. This was used at first in small amounts as a catalyst for acetylation but was abandoned in later experiments. This intensified he resorcinol-fuchsin staining to such an extent that even some collagenous fibrils tenaciously retained the stain. This occurred when sulfuric acid was added to control solutions as well as when it was used as a catalyst in the experiments with acetic anhydride.

COMMENT

The first three sets of data with which I am concerned here require little comment, for they are in accord with prediction based on previous studies.

First, all previous studies had been conducted on materials introduced into the subcutaneous tissues. Data have been presented in this report which show that the transformation in question occurs in various other sites in the body. There were many minor variations in the rate and end result of the transformation in the different localities. These will not be discussed, for they have no immediate bearing on the problem. Suffice it to say that insoluble products of the conversion were always either in the intercellular phase or within the cytoplasm of phagocytic cells and never within the cytoplasm of epithelial cells.

The second set of data proves that mammalian tissues contain ether-soluble or acetone-ether-soluble materials which undergo the transforma-

tion. These materials were found in each tissue under investigation, namely, human brain, human blood, guinea pig brain and guinea pig liver. The products of transformation were either granular or globular and were not abundant in any instance. They elected the Ziehl-Neelsen and the resorcinol-fuchsin stain, as demonstrated previously with the products of transformation of unsaturated compounds from non-mammalian sources. Further manipulation of tissue extracts, with eventual isolation of the more highly unsaturated components, would certainly improve the results of this study, and the findings might be of considerable interest.

The third set of data aids in a further naturalization of the biologic transformation under discussion. Previous investigations have principally been restricted to esters of unsaturated long chain organic acids with monohydric alcohols or glycerol. The data presented here again demonstrate the importance of the acyl radical, for cholesterol esters yielded products of transformation similar to those previously obtained by the use of oxygenated methyl esters of the same group of unsaturated acids. There was evidence of partial hydrolysis of cholesterol esters during their residence in the intercellular region. Therefore, there was no absolute proof that cholesterol esters as such were transformed, although if past experience with other esters can be called on for support of an opinion, the direct conversion of cholesterol esters independent of hydrolysis probably occurred. There was little doubt that some mild oxygenation of the unsaturated acyl groups accompanied preparation of the esters; it is not entirely proper to state that continuous membranes formed at cholesterol ester–tissue interfaces in the absence of preliminary oxygenation such as was necessary for membrane formation by methyl esters of the same group of unsaturated acids. This may serve as an object for further study because of the general similarity of transformations of highly unsaturated liquid cholesterol and methyl esters.

The purified egg lecithin introduced in a variety of solvents yielded no insoluble material which was stained positively by the Weigert or the Ziehl-Neelsen method. The result is contrary to prediction. There is no explanation for the negative result. Perhaps more highly unsaturated natural lecithins, such as those obtainable from liver or brain, might be more satisfactory products for study.

Microscopic studies showed frequent participation of protoplasm in the basal structure of the membranes at the lipoid-aqueous interface. The proteins which were demonstrated as definitely contributing to the basal structure of some areas of continuous membranes were collagen and fibrin. The implication of proteins seemed to depend principally on restricted interpermeation of lipoids and proteins at the interface as well as on surface adhesion. The interpermeation was illustrated by the presence of delicate films of lipoid, which extended from the inter-

facial region to pass between limiting strands of collagen and fibrin. The adhesion of well defined proteins to the altered lipoids of the membranes was everywhere in evidence and usually was independent of demonstrable interpermeation. This appeared to be of the nature of a strong surface attraction, although the chemical binding of altered lipoids to limiting proteins at the interface must be considered as a likely possibility.

The implication of the marginal protoplasm of mesenchymal cells at the interface in the basal structure of membranes presents some interesting problems. The conclusion that cytoplasm may be fused with the altered lipoids arose from two morphologic facts. First, in rare instances no sharp line of separation between marginal cytoplasm and adherent lipoidal membranes could be distinguished. Second, lines of intracytoplasmic cleavage were occasionally noted where membranes in the course of mechanical separation from their site of formation had been detached. In these areas marginal cytoplasm remained intimately fused with the detached membranes. Thus, in several instances an effective marginal cytoplasmic block was produced by some interaction between cytoplasm and adjacent altered lipoids. This is emphasized not only because it is illustrative of the participation of protoplasm in the basal structure of portions of membranes but because it may be a useful experimental device for studying controversial questions related to histogenesis of intercellular materials at cell margins.

Another aspect of the participation of tissue proteins in the structure of membranes was a consequence of the tendency of the membranes to assume a folded or wavy contour. Under these circumstances the tissue element involved at the interface accompanied the convolutions. As the amplitude of the folds was increased and as the distance between their crests was decreased by lateral condensation, the protoplasmic component —fibrin, collagen or cytoplasm—became attenuated and normally oriented to the general plane of membrane boundary. With continued condensation the delicate protoplasmic invaginations became attenuated to the point of invisibility. The fate of the proteins so included could not be determined. As a rule, the prolongations retained a basal connection which in some instances was obliterated either by compression or by detachment of membranes from their site of formation. This mechanism gives further insight into the physical possibilities of interfacial behavior under biologic circumstances. With large numbers of thin films of this general type, the surface forces would be great indeed and conducive to high elasticity and tensile strength. From this model many variations depending on the distribution of the lipoid with respect to the protein are of course realizable, and these data give only the simplified relations which exist between a continuous lipoidal film and a limiting, more or less continuous protein film.

It has been shown previously that methyl esters of unsaturated acids of cod liver oil as obtained by high vacuum distillation were not transformed into long continuous membranes at lipoid-aqueous interfaces in the tissues. If the esters were oxygenated at room temperature for several days, they acquired the property by which they were amenable to transformation into insoluble continuous membranes at the interface between residual liquid-soluble compounds and the aqueous limiting tissue phase. It has been accepted for some time that such treatment of highly unsaturated long chain acyl groups results in the addition of oxygen at the double bonds with the formation of peroxides.[4] These peroxides are unstable and are supposed to rearrange into hydroxy-ketone structures which have a relation in equilibrium. Insoluble products which often arise in vitro as a consequence of oxygenation of such highly unsaturated acyl groups are assumed to be either a consequence of association which depends on the presence of a hydroxy-ketone structure or a consequence of chemical condensation in which a molecular union occurs across oxygen bridges.

This oxidative process is inhibited in vitro by the presence of such potent antioxygenic agents as hydroquinone and pyrogallol.[5] Therefore, on the assumption that similar oxygenation and condensation were responsible for the biologic transformation, an attempt was made experimentally to inhibit the transformation in vivo by adding hydroquinone or pyrogallol to unsaturated compounds prior to introduction into the tissues. The presence of these reagents, contrary to prediction, had no influence on the biologic transformation. Experiments of shorter duration might show retardation, but this does not seem likely in view of the data.

The addition of oxygen to unsaturated compounds such as those under discussion is greatly accelerated in vitro by the presence of a trace of iron or several other metals in the materials exposed to oxygen.[4] As a consequence, the rate of uptake of oxygen and the formation of insoluble materials are increased. On the assumption that iron would have a similar action in vivo, several iron salts were added to various unsaturated materials prior to injection. The results indicated clearly that, in accord with prediction, the presence of iron was effective in accelerating the biologic transformation.

The preceding results, together with previous proof that preliminary oxygenation of highly unsaturated methyl esters was required before they were convertible into membranes under biologic circumstances, encouraged a further investigation of molecular changes which are responsible for some of the morphologic findings. The experiments

4. Coffey, S. J.: J. Chem. Soc. London **119**:1152 and 1408, 1921.
5. Mattill, H. A.: J. Biol. Chem. **90**:141, 1931.

which showed that the unsaturated compounds lost their initial capacity to reduce perosmic acid as they became transformed into membranes gave further impetus to a consideration of the molecular structure of the membranes.

Attention was first concentrated, therefore, on demonstrable chemical alterations brought about by oxygenation of unsaturated methyl esters in vitro, for this treatment was required before these compounds were amenable to transformation into membranes. Two chemical alterations were demonstrated by isolation in vitro of crystalline phenylhydrazones and benzoyl derivatives. From such data one may conclude that hydroxyl and keto groups were formed during the period of oxygenation. This is in keeping with prediction. These data afforded a logical basis for an investigation of the insoluble products which were formed under biologic circumstances.

The presence of such groups in the mature membranes could not be proved by any direct means. The insolubility of the materials and the small amounts available for study presented major difficulties from the chemical point of view. The only alternative was to make the determinations on the materials in thin sections of tissue with the hope, first, that the chemical reagent might react at least with surface molecules and, second, that an appropriate method for detecting the presence or absence of reaction might be devised. It was a simple matter to make the tests but a more difficult one to devise a method for determining whether reaction had occurred. On the thesis that the presence or absence of reaction might be measured by an interference in the election by membranes of some stain, the resorcinol-fuchsin stain was employed. This indirect method was crude and not wholly adequate. However, I believe that this is an appropriate procedure and that within limits interpretation of the data is permissible. Better methods, however, must be devised before any considerable degree of precision can be obtained.

It is not necessary at this time to discuss the addition of the "specific" reagents, as their vagaries are not thoroughly understood. This is especially true when the compounds undergoing reaction have conjugated keto groups or keto-enol arrangements, which are distinct possibilities in the structure of the altered compounds in the tissues.

The deductions from the experimental data, subject to the preceding argument, are as follows: First, reactions between the reagents and the altered lipoids in thin sections of tissue do occur. Second, the reactions with the "keto-fixing" reagents create a state in which there is a constant decisive inhibition of the capacity of membranes to elect Weigert's resorcinol-fuchsin stain. From these data the deduction is drawn that the inhibition is a consequence of reaction with keto groups in the membranes. Third, the reactions with two reagents which characteristically condense with hydroxyl groups create a state in the

membranes by which a slight inhibition of their capacity to elect Weigert's stain is detectable. From these data the inference is drawn that reactive hydroxyl groups are present in the membranes. Fourth, that if successive determinations are made, first with a "keto-fixing" reagent and second with a "hydroxyl-fixing" reagent, the inhibitory effect produced by the "keto-fixing" reagent is reduced rather than enhanced. No explanation for this result can be given. It is contrary to prediction, but the reasons for the discrepancy may not be wholly referable to technical difficulties. These data give no cause for quantitative deductions, because the mode of action of the resorcinol-fuchsin stain is unknown and it is possible that further experimentation with other "hydroxyl-fixing" reagents will yield a more satisfactory inhibition. A real difficulty, of course, lies in the fact that even though a large number of reactive hydroxyl groups condense with a reagent, the staining reaction might not necessarily be influenced in any way. The fact that the election by elastic tissue of the resorcinol-fuchsin stain was not interfered with by any reagents except sulfuric acid adds to the complexity of the interpretations. However, it is hoped that these specific reagents, which are of great value in organic chemistry, will be of further use in histochemistry and that some of the puzzles which have arisen during this investigation of the chemical structure of these membranes will be solved.

SUMMARY

Previous investigations have shown that several liquid soluble unsaturated organic compounds become insoluble during a period of residence in the subcutaneous tissues of guinea pigs and that the insoluble compounds may be oriented to form continuous membranes at lipoid-aqueous protoplasmic interfaces. In the studies reported here, this biologic transformation has been investigated in a sufficient number of localities to permit the hypothesis that it occurs in general in the intercellular medium.

The liquid soluble organic compounds utilized in previous investigations were not obtained directly from mammalian tissues. In this report it has been proved that compounds amenable to the transformation in question are present in ether or acetone-ether extracts of mammalian liver, blood and brain. A further naturalization of the process was achieved by demonstrating that cholesterol esters of highly unsaturated acids are susceptible to the transformation and that the insoluble products are often oriented to form continuous membranes at cholesterol ester–aqueous protoplasmic interfaces. Purified egg lecithin, on the other hand, did not yield the slightest trace of insoluble products.

Previous studies have indicated that partial biologic oxidation and subsequent polymerization of incompletely oxidized unsaturated compounds were the principal chemical mechanisms on which the trans-

formations depended. Additional support of this thesis has been obtained by investigations discussed in this paper. These studies have shown, first, that several iron-bearing compounds accelerate the transformation in vivo; second, that as the unsaturated compounds undergo conversion, they gradually lose their initial capacity to reduce perosmic acid; third, that preliminary oxygenation of certain highly unsaturated methyl esters is necessary before these are convertible into long continuous membranes and that an accompaniment of this treatment is the creation of hydroxyl and keto groups in some oxygenated molecules, and fourth, that these groups, especially keto groups, seem to be created in the process of membrane formation in the biologic environment.

Although the membranes are composed principally of altered unsaturated compounds, the morphologic observations here reported show clearly that protoplasm is at times implicated in the basal structure of membranes in the lipoid-aqueous interfacial zone. The participation of proteins such as collagen, fibrin and marginal cytoplasm in the membrane structure may be a consequence of purely physical factors, but the data do not disprove actual chemical lipoprotein condensation.

Therefore, it is necessary to deal with long normal chain highly unsaturated acyl groups which acquire in this semibiologic system carboxyl, hydroxyl and keto polar groups on hydrolysis or partial oxidation, which associate to form insoluble long continuous membranes at lipoid-aqueous protoplasmic interfaces and which merge with protoplasmic elements at such interfaces to form lipoprotein structures. These structures, as they mature, eventually lose all characteristics by which their chemical origins might conveniently be proved.

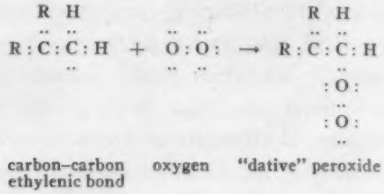
APPENDIX

Most compounds used in these investigations belong to a general class of organic substances which undergo auto-oxidation and polymerization in vitro. Milas[6] has summarized the data of organic chemistry relevant to this subject. It may be worth while to clarify the plan and principles which underlie the present investigation by a brief discussion of the theory of auto-oxidation and polymerization of auto-oxidizable compounds.

Oxygen may exist in many states. In some states it is highly reactive and in other states relatively inert. Molecular atmospheric oxygen is a relatively inactive form of oxygen and most compounds are indifferent to its presence. Other compounds, classified in general as auto-oxidizable compounds, will readily react with this form of oxygen. In the compounds used in the present experiments the ethylenic linkages in the long carbon chains of acids or acyl groups are the important reactive centers. One molecule of oxygen may add to each of the reactive double bonds. The oxygenated compound thus becomes a "dative" peroxide. The oxygen in this compound exists in a highly reactive state and

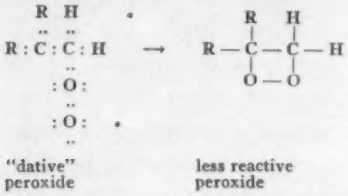
6. Milas, N. A.: Chem. Rev. **10**:295, 1932.

may enter into reactions which were not possible prior to its addition to an ethylenic linkage. A "dative" peroxide may be represented electronically as follows:



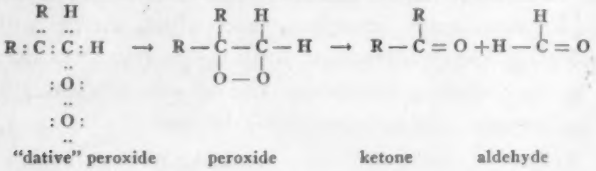
carbon–carbon ethylenic bond | oxygen | "dative" peroxide

This compound as a rule exists only in theory. In general it is unstable and represents the highest level of oxygen "activation." The reaction just illustrated may be reversible or irreversible. If reversible, the oygen is released and the carbon–carbon ethylenic bond is regenerated, becoming again susceptible to oxygenation. If the reaction is irreversible, the "dative" peroxide may undergo one of several changes. It may undergo rearrangement to form a relatively stable less reactive peroxide, which may be represented as follows:



"dative" peroxide | less reactive peroxide

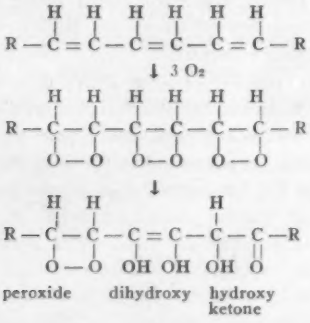
It may decompose to form an aldehyde and a ketone as follows:



"dative" peroxide | peroxide | ketone | aldehyde

The aldehyde and ketone are in turn available for further oxidation.

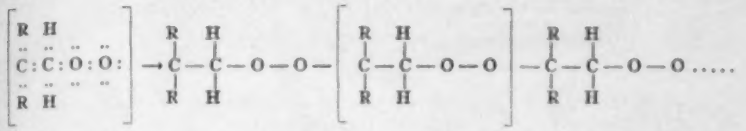
When dealing with ethylenic linkages in a chain of carbon atoms, a possible sequence, which is supported by experimental observations, may be given as follows (the hypothetic "dative" peroxide is omitted from the formulation):



peroxide | dihydroxy | hydroxy ketone

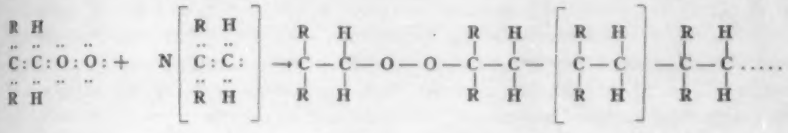
This illustrates the possibility of keto-enol tautomeric rearrangements within single molecules and the creation of reactive polar groups along hydrocarbon chains.

The polymerization of long chain highly unsaturated auto-oxidants is a complex process and is not well understood. There is some evidence, however, which indicates that "dative" peroxides may polymerize with similar peroxides to form compounds of high molecular weight. The schematic representation of this process is as follows:



N-2

The "dative" peroxide may polymerize with other auto-oxidant molecules which have not added oxygen. In this instance a chain reaction may occur in which polymerization of the unoxygenated auto-oxidant is the only significant event. This may be shown by the following formula:



N-2

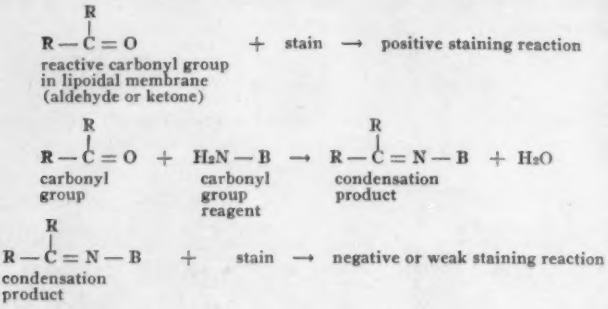
In the course of the study of the behavior of complex auto-oxidant molecules in the tissues of animals where little free oxygen is supposed to exist, the thesis is held that at least some similarities exist between the behavior of these auto-oxidants in vitro and in vivo. In general, there can be little doubt, in view of the evidence, that polymerization of auto-oxidant molecules progresses smoothly in the vital system under investigation. The process is more rapid in the biologic system than under uncatalyzed conditions of oxygenation within the biologic temperature range in vitro. It seems logical to deduce that some biologic mechanisms are responsible for catalyzing the process in the tissues and for transporting oxygen to the lipoidal substrate.

At first glance, it would seem a hopeless task to inquire into the more minute structure of the interesting membranes which form at the lipoid-aqueous protoplasmic interfaces. If any information is to be gained, microhistochemical methods are required.

It has been established by previous workers that the black staining of lipoidal compounds by perosmic acid is referable to the presence of double bonds in the long carbon chains of the lipoids. The black staining is attributed to the impregnation of the lipoids with black reduction compounds of perosmic acid. This histochemical reaction therefore becomes of use in these studies, for it illustrates that lipoidal compounds under the conditions of these experiments may gradually lose an initial capacity to reduce perosmic acid. This evidence indicates that at least a majority of the ethylenic linkages have disappeared, presumably in part by reaction with oxygen.

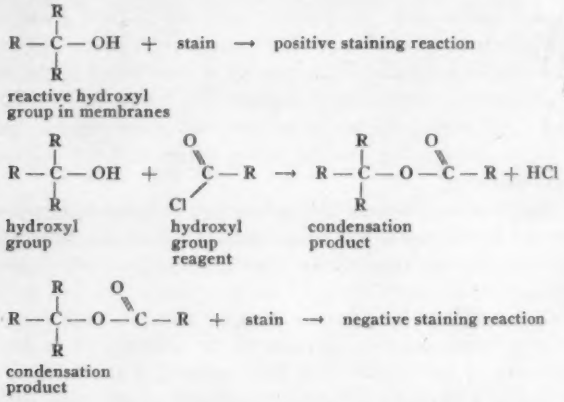
The proof that oxygen is implicated in satisfying available ethylenic linkages during the biologic transformation of lipoidal compounds into membranes must necessarily rest on a satisfactory demonstration of oxygen which is bound in the lipoidal membranes. Functional oxygen-containing groups, such as peroxide groups, carbonyl groups and hydroxyl groups, represent reactive centers for study, if the formulation given is logically followed.

For investigating the possible presence of available carbonyl groups which will react with carbonyl group organic reagents, the following scheme was devised for use on thin sections of tissue:


reactive carbonyl group in lipoidal membrane (aldehyde or ketone)
+ stain → positive staining reaction
carbonyl group
carbonyl group reagent
condensation product
+ H₂O
condensation product
+ stain → negative or weak staining reaction

An appropriate stain which was useful as a detector of this reaction proved to be Weigert's resorcinol-fuchsin stain. In other words, membranes which normally elect the resorcinol-fuchsin stain fail to elect this stain if they have been previously treated with reagents which characteristically condense with reactive carbonyl groups. This is presumptive evidence that some compounds in the membranes possess reactive carbonyl groups and that these groups may be primarily responsible in this instance for the positive staining of the membranes by the resorcinol-fuchsin method.

A similar scheme was devised for demonstrating reactive hydroxyl groups in the lipoidal membranes. This may be formulated as follows:



Thus far, attempts to demonstrate reactive hydroxyl groups in compounds of which the membranes are composed have not been very successful. This may be due to one of several factors: First, reactive hydroxyl groups, if present, may not be available in sufficient numbers. Second, the reagents may not react with the groups which are present. Third, an appropriate staining reaction which depends on the presence of reactive hydroxyl groups has not been used. The third possibility is most likely, and a further study of this problem is indicated. Other projects include a search for peroxide and keto-enol groups. Finally, the problem of possible actual chemical conjugation of tissue proteins with the altered lipoids adds further complications to the study. This problem must be attacked primarily by synthetic methods, controlled by histologic observations.

TRANSPLANTATION OF TOOTH GERM ELEMENTS TO MARROW CAVITIES OF TIBIAS OF KITTENS

CHARLES J. SUTRO, M.D.
AND
LEO POMERANTZ, B.S.
NEW YORK

The fact that occasionally a tumor resembling an adamantinoma develops in the human tibia prompted this study of autogenous tooth germ elements transplanted into the marrow cavities of tibias of kittens. We were particularly interested in the immediate histologic changes taking place in the transplants.[1]

Six kittens, each weighing about 1 Kg., were anesthetized with sodium amytal. An unerupted canine tooth was exposed by excision of a thin plate of jawbone and removed. The calcified dentin and enamel were discarded. The soft tissue of the tooth was placed in warm Ringer's solution. The membranes were halved longitudinally; one half was discarded and the other placed in the upper third of the tibial marrow cavity.

The site for reception of the transplant was prepared as follows: The periosteum on the medial surface of the tibia was retracted. Several small drill holes were made in the cortex, outlining a small rectangle of bone. This rectangular shell was removed, a small amount of marrow substance was curetted, and the tooth membranes were placed in the resulting cavity. The periosteum was then sutured so that the cortical defect was covered over.

In some of the animals a similar procedure was pursued at a later date with the opposite unerupted canine tooth and the opposite tibia. In several cases one of the legs containing a transplant was amputated after a short interval to permit study of the early changes.

Individual transplants were left in situ for three, twenty-one, thirty-one, thirty-one, fifty-one, one hundred and thirty-one, one hundred and sixty-two, one hundred and sixty-six and one hundred and eighty days, respectively, and then removed for examination.

After a three day period, most of the pulp cells were found flat; some were tricornuate in general shape and had fine terminal filaments. At the periphery of the pulp mass there were thin sheets of nonactive

From the Laboratory Division of the Hospital for Joint Diseases.

1. Wolfert, B., and Sloane, D.: J. Bone & Joint Surg. **20**:1011, 1938.

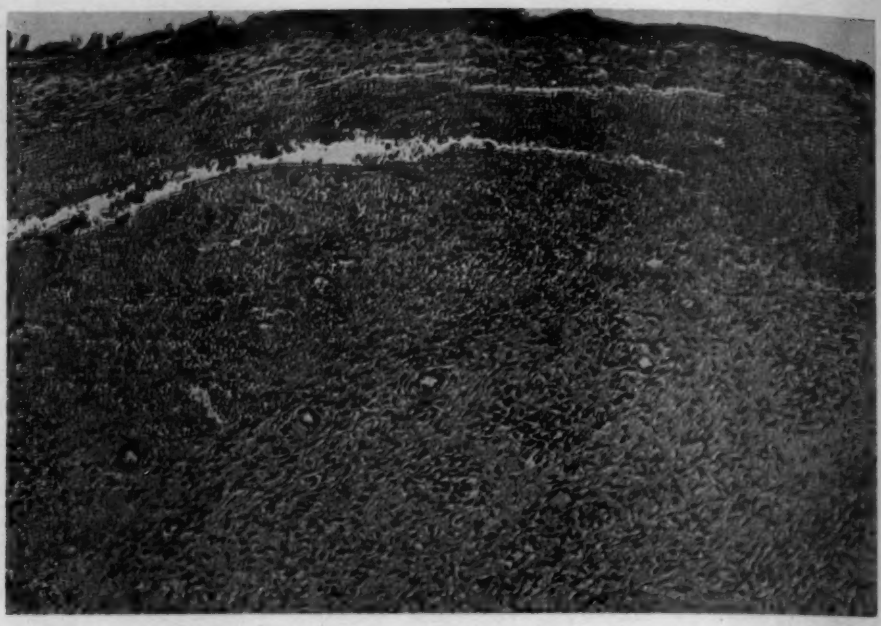


Fig. 1.—Photomicrograph of transplanted dental pulp after a three day interval; × 100. Note the viable pulp cells as well as the areas of necrosis at the margin. At the extreme periphery is a thin layer of epithelial cells, most probably ameloblasts.

Fig. 2.—Photomicrograph of a transplant after a twenty-one day interval; × 100. Note the active islands of dentin formation.

ameloblasts. No evidence of active formation of dentin or of enamel was noted (fig. 1).

In another transplant, examined twenty-one days after implantation, the active formation of dentin was the most striking feature (fig. 2). The pulp cells had hypertrophied, were lined up in a regular fashion and adjoined the predentin zone. Interspersed among the fibrils of the pulp matrix were numerous amorphous calcareous masses, apparently

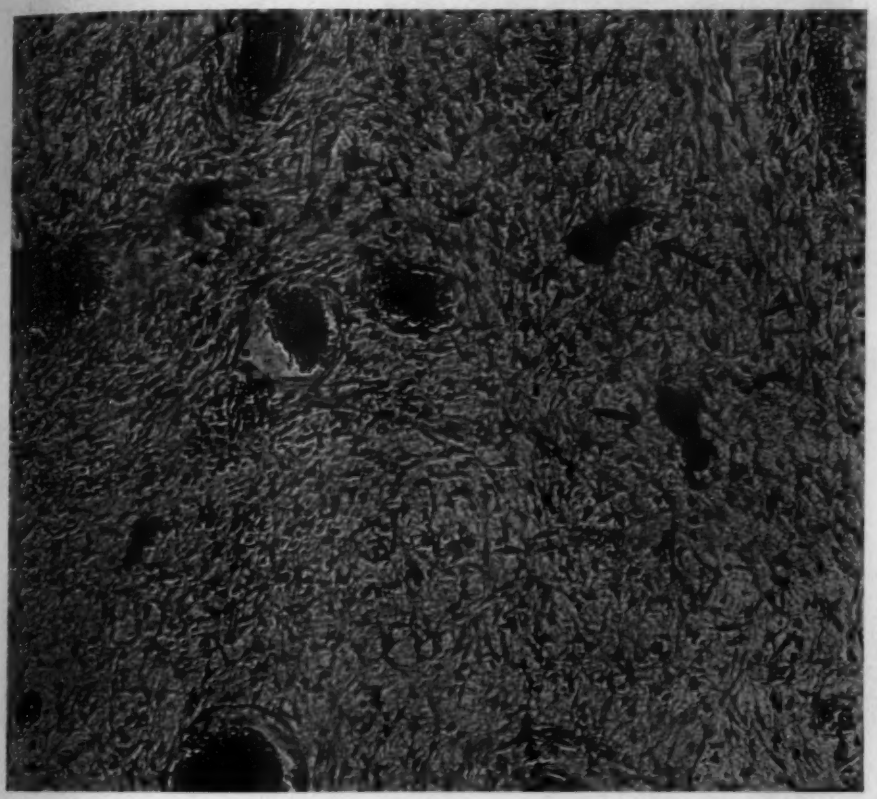


Fig. 3.—Photomicrograph of a twenty-one day transplant; × 100. The living pulp shows several amorphous calcospherities (arrows).

similar to the calcospherites found in normal dentin (fig. 3). No new enamel or islands of ameloblasts were noted.

After thirty-one days, a considerable amount of dentin was present. A good portion of it was acalcareous. In other parts of the transplant, small and large segments of Hertwig's membrane were encountered. Differentiation could be seen to have been progressing. In still other

Fig. 4.—Photomicrograph of a thirty-one day transplant, revealing activity of Hertwig's membrane in the pulp tissue; × 50. Note the island of ameloblasts lying outside the pulp tissue (arrow) and showing no enamel formation.

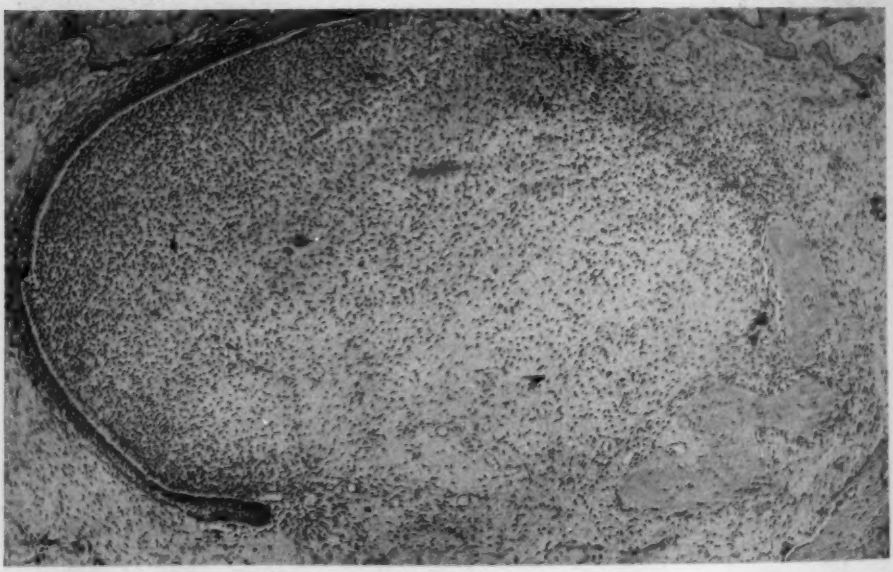


Fig. 5.—Photomicrograph of a thirty-one day transplant, showing a large segment of Hertwig's membrane with early differentiation; × 65. Note the cellular proliferation of the pulp near the membrane.

portions of the transplant there were islands consisting of aggregate masses of epithelial cells; there were nests of ameloblasts (fig. 4).

In another transplant examined after thirty-one days, several large islands of epithelium were observed. Each presents definite evidence of an early attempt at formation of both enamel and dentin. It is of special importance that where the islands of epithelium were not in contact with pulp there were no signs of enamel formation (figs. 4, 5 and 6).

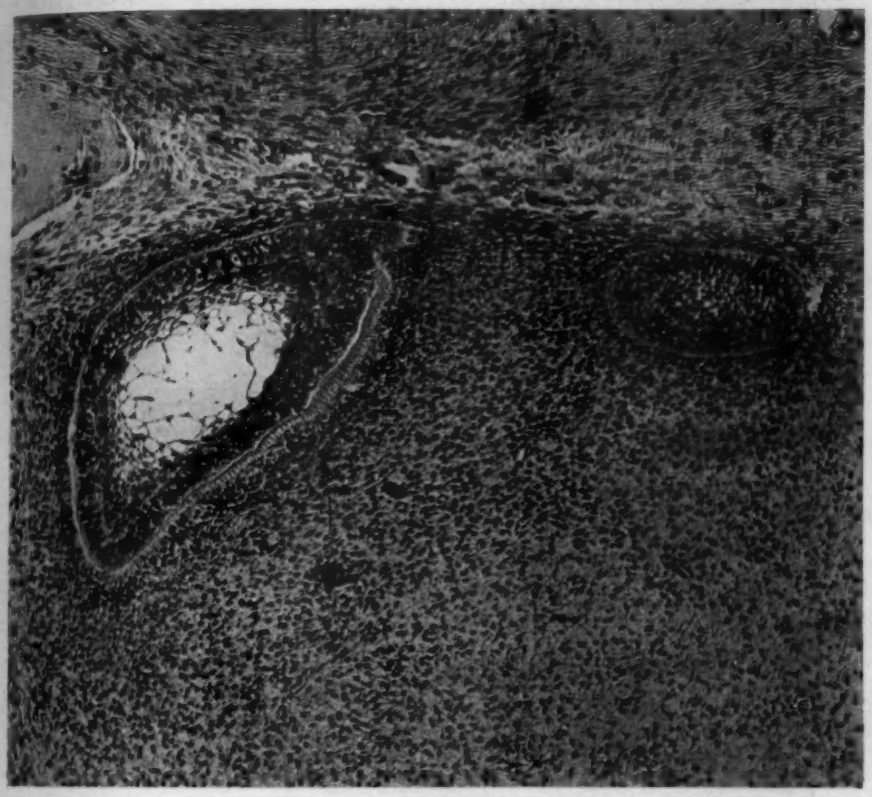


Fig. 6.—Photomicrograph of a thirty-one day transplant showing two islands of proliferating ameloblasts in the pulp; × 100. Note that the contiguous pulp cells are being transformed into odontoblasts.

When new bone was being formed near the transplanted dental pulp, the osteoblasts were unusually large, some showing mitosis and others forming giant cells (fig. 7).

Nothing very unusual was found in the tibia after the thirty-one day period. Dentin formation had continued, and most of the pulp

had been crowded out by the newly formed bone. One preparation (fig. 8) revealed a large area of osteodentin in process of formation.

COMMENT

This study shows in the first place that autogenous tooth germ elements transplanted into the tibial marrow can regenerate. Hertwig's

Fig. 7.—Photomicrograph of a thirty-one day transplant showing new bone formation near active Hertwig's membrane; × 85. Note the unusually large dark staining osteoblasts (arrow).

sheath survived and proliferated in its new environment. Special interest is attached to the finding that when the enamel and odontoblast layers were in proximity both showed signs of continued activity. In places

where ameloblasts were absent the transplanted odontoblasts did not show continued activity, merely forming irregular sheets of dentin. Furthermore, the pulp cells did not differentiate into odontoblasts when ameloblasts were not present nearby. On the other hand, where ameloblasts were present without adjoining pulp cells or odontoblasts, they merely became transformed into islands of epithelial tissue.[2]

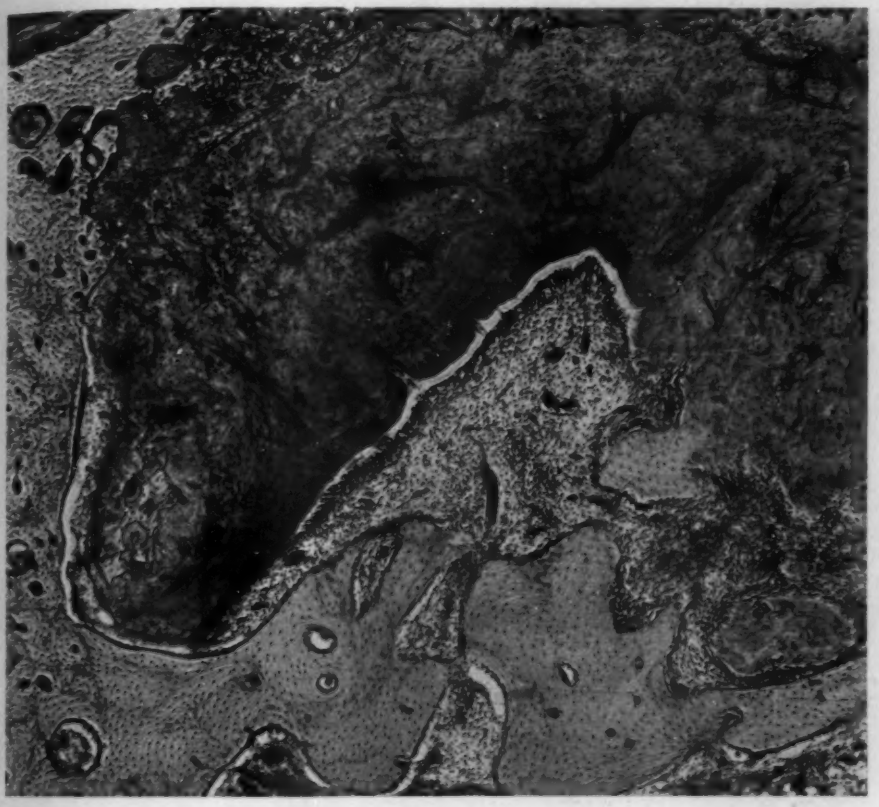


Fig. 8.—Photomicrograph of a one hundred and sixty-two day transplant showing a large mass of osteodentin; × 45. Note zone of predentin with a layer of odontoblasts.

If these observations are applicable at all in the interpretation of the so-called adamantinoma of the human tibia, they suggest that this tumor may be an ameloblastoma rather than an odontoblastoma (adamantinoma). If this tumor thus grows out of misplaced tooth germ, the

2. Legros, C., and Magitot, E.: Compt. rend. Acad. d. sc. **78**:357, 1874. Huggins, C. B.; McCarroll, H. R., and Dahlberg, A. A.: J. Exper. Med. **60**:199, 1934. Glasstone, S.: J. Anat. **70**:260, 1936.

observed lack of formation of enamel or of dentin in connection with it would be due to the absence of odontoblasts or pulp cells adjacent to the ameloblasts.[3]

SUMMARY

Tooth germ elements of kittens regenerate after autogenous transplantation into the tibial marrow cavity. Which of the various germ elements will proliferate and to what extent depend on the degree of preservation of the enamel-dentin relationship.

3. Robinson, H. B. G.: Arch. Path. **23**:664 and 831, 1937.

SOLID TO CYSTIC DEGENERATION IN AN AMELOBLASTOMA

HAMILTON B. G. ROBINSON, D.D.S., M.S.
ST. LOUIS
AND
W. R. J. WALLACE, D.D.S.
ROCHESTER, N. Y.

From histologic studies Kronfeld[1] concluded that "the cystic adamantinoma develops from the solid type by cystic degeneration of the stellate reticulum." Robinson,[2] commenting on a histologic study of 4 cases, said, "While no single ameloblastoma has yet been traced from solid to cystic form through histologic studies, it seems highly probable that such a transition takes place. . . . While this evidence is highly suggestive, it cannot be claimed that such a process is known to take place." This opinion is confirmed by a review of the literature.[3]

This histologic evidence seems to indicate that the ameloblastoma begins as a homologue of the developing tooth and follows the general structural development seen in the stages of odontogenesis up to the point of function (laying down of enamel). From that point on the neoplasm follows a degenerative course leading to the formation of a multicystic tumor. The degeneration appears to occur in the stellate reticulum-like cells in the center of the follicle. This mode of transition leaves the ameloblastoid columnar cells as a cystic lining, although these cells may be compressed to a cuboid or squamous form.[2]

In the previous histologic study of ameloblastoma[2] a solid type was described as resembling basal cell carcinoma, and a photomicrograph of it was used as an illustration of early cystic change in the follicles. After the publication of that report the patient returned with a huge tumor of the mandible. The case history follows:

A man 49 years of age, married, said that about one year prior to examination he noticed a hard painless nodule, on the buccal surface of the right mandibular ridge. It grew slowly until about two months before presentation, when it sud-

From Washington University School of Dentistry, St. Louis, and the University of Rochester, Rochester, N. Y.

This work was supported in part by grants from the Carnegie Corporation of New York and from the John and Mary Markle Foundation.

1. Kronfeld, R.: J. Am. Dent. A. **17**:681, 1930.
2. Robinson, H. B. G.: Arch. Path. **23**:664, 1937.
3. Robinson, H. B. G.: Arch. Path. **23**:831, 1937.

denly became swollen and painful. Two days later a bloody discharge was noticed about the mandibular right second molar tooth. This disturbance was repeated in about two weeks' time. The mandibular right second molar tooth was extracted. On examination the growth appeared as a nontender, painless hard nodule. A biopsy was made, and the growth was reported as ameloblastoma. At operation the cyst was worked free from the bone, care being taken to preserve the structure of the bone. The tumor mass was split and delivered in small pieces. The area was curetted and the inferior dental artery ligated. The cavity was cauterized with phenol and washed with alcohol.

The gross specimen consisted of several small pieces of tissue of irregular shape, with fragments of adhering bone. The cut surface was smooth, glistening, dense and white, with several smooth red areas, which probably represented cysts.

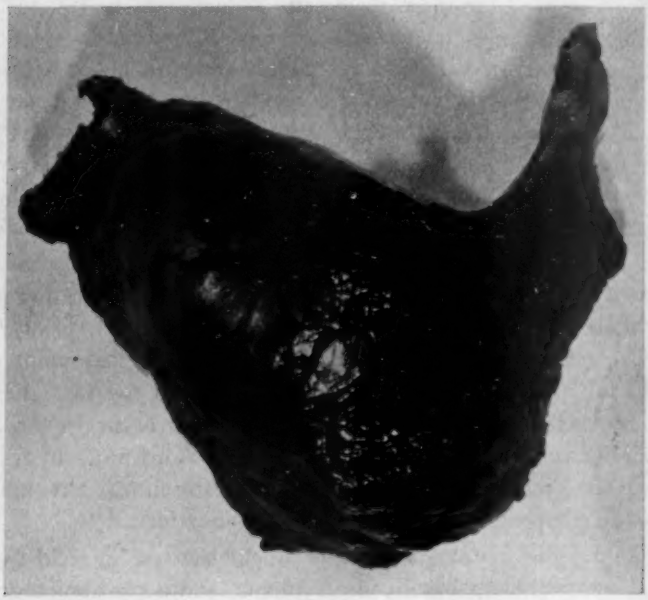


Fig. 1.—Mass removed at operation. The size of the tumor mass with the attached bone was 8 by 6 by 6 cm.

Microscopically, columns of epithelium were found in connective tissue stroma. The epithelium was of columnar type, with a delicate basement membrane and more irregular epithelial cells between. In places the intercolumnar cells suggested stellate reticulum, and in still other areas small cysts containing a homogeneous pink-staining material were found. While epithelial pearls were not seen, the appearance of some of the cysts suggested a tendency toward keratinization. The epithelium of the neoplasm showed connection with the oral mucous membrane.[2]

The patient failed to return for further study and possible partial resection, as ordered. Five years after the last report he presented a huge swelling of the right mandible and submaxillary area. Roentgenographically, destruction of the

bone was demonstrated in areas extending from the symphysis to the angle. The mass was not painful; it was slightly fluctuant and suggested lobulation. At operation the neoplasm and mandible were exposed through an external incision, and the bone was sectioned just beyond the symphysis on the left and posterior to the angle on the right.[4] The removed mass is seen in figure 1.

Sections were made through the entire tumor mass and the attached mandibular bone. Figure 2 shows one of these sections. Cysts may be seen in it, ranging from microscopic size to 4 by 4.5 cm. The cysts are lined by epithelial cells of cuboid, columnar and squamous type, and the centers of the cysts are occupied by mucoid material in droplets and solid sheets with occasional cholesterol slits. In some areas the bone has disappeared, while in others evidence of growth pressure is evidenced by osteoclastic activity on the cyst side and osteoblastic activity on the other. There are large areas in which solid strands and columns of

Fig. 2.—Photomicrograph of a cross section of the entire mass, showing a cystic area and bone; × 2.5. Note the large and small cysts, the areas of bone and the solid epithelial strands and mucoid-like material in the cyst cavities.

epithelial cells remain. These resemble the areas seen at the previous operation,[5] with columnar cells externally and more irregular or stellate reticulum-like cells centrally (fig. 3*A*). The epithelium lining the cysts, although it may be compressed to squamous form, is still capable of differentiation and proliferation. In figure 3*B* an area from the cyst wall is seen with proliferation of the epithelium and differentiation toward the form resembling the dental lamina. Early cystic change is seen even in this epithelium. In the same area, adjacent to this active proliferation of epithelium, osteoclastic activity is seen. The stroma, not the epithelium, is rich in blood vessels.

4. The operation was done by Forrest Young and W. R. J. Wallace.

5. Robinson,[2] figure 2*A*.

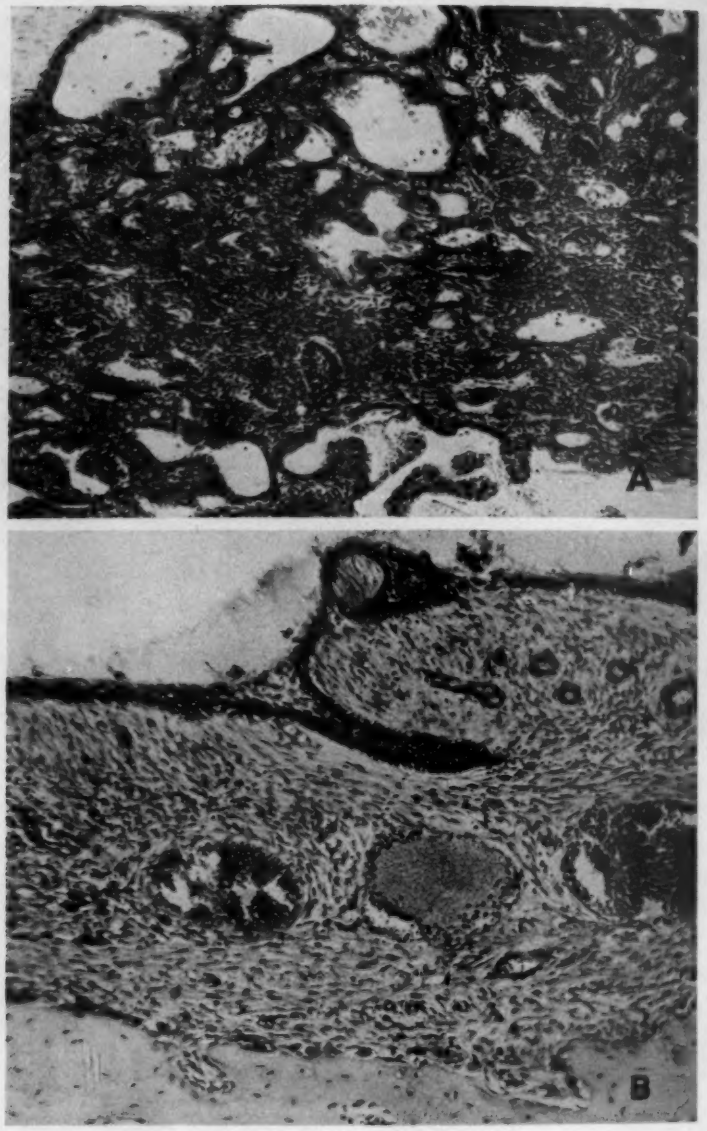


Fig. 3.—*A*, area from figure 2 at higher magnification, showing epithelial strands with columnar cells externally and stellate cells centrally. Note early cystic degeneration. *B*, area from figure 2 at higher magnification, showing the proliferative power of the cyst lining. Note the resemblance of the epithelial strand to dental lamina, the intraepithelial cyst formation and the osteoclastic activity at bone adjacent to the area of epithelial growth.

COMMENT

The solid to cystic change in the ameloblastoma previously demonstrated in series of cases was not corroborated by clinical studies or by a microscopic study of these changes in a single case. The present case would not be of great value as an isolated example, but coupled with the laboratory series it can be presented as strongly confirming the previous indications. It is rather interesting that the solid phase of this identical neoplasm was used as an example of a solid ameloblastoma showing an early cystic tendency.

The demonstration that the ameloblastoma may degenerate from solid to cystic form should be of value to the surgeon. If the tumor mass can be removed in its solid stage, enucleation and cauterization may suffice, for it will not yet have extended its follicular growth between the bone trabeculae. At a later date the cystic transition may have begun, and the many strands and cysts will be growing by pressure into the bone spaces. At this stage enucleation is difficult or impossible, and resection becomes the operation of choice. Ivy and Curtis[6] recommended primary resection probably because the surgeon seldom sees the ameloblastoma in its early painless and slow-growing stage. The responsibility falls on the clinician, who must make the diagnosis early.

SUMMARY

A case of multicystic ameloblastoma is presented. Five years previously a growth of this neoplasm was removed and was a solid tumor. This change is strong confirmation of previous laboratory demonstrations that the ameloblastoma changes from a solid to a cystic stage through degeneration of the stellate, reticulum-like cells.

6. Ivy, R. H., and Curtis, L.: Ann. Surg. **105**:125, 1937.

ATTEMPTS TO PROPAGATE FOWL TUMORS PRODUCED BY BENZPYRENE AND BY VIRUS

SITES OF IMPLANTATION USED: EYE OF CHICKEN AND CHORIOALLANTOIC MEMBRANE OF CHICK EMBRYO

SIDNEY ROTHBARD, M.D.
AND
JOHN R. HERMAN
NEW YORK

A transmissible spontaneous fowl tumor was described by Rous[1] (1910), and in the following year he showed that it could be transmitted with either cellular material or cell-free filtrate.[2] Rous and Murphy[3] propagated this tumor by introducing a cell-free filtrate onto the chorioallantoic membrane of the chick embryo. Subsequently several different "filtrable tumors" were described by other investigators. Since chicken tumors can be produced readily by application of tar, the question arises whether or not the tumors induced by a chemical contain a virus.

Murphy and Landsteiner[4] were the first to investigate this problem. They produced spindle cell sarcoma in 2 chickens by injections of coal tar. One of the tumors was transmitted by cellular material for 11 generations and proved to be highly invasive. These investigators were unable at any time to transmit the sarcoma with a cell-free filtrate or a desiccate. Peacock[5] induced fowl sarcoma by injections of tar and of 1,2,5,6–dibenzanthracene-lard mixtures and likewise failed to transmit the tumors with cell-free material. McIntosh[6] produced sarcoma by injecting tar, and, contrary to Murphy and Landsteiner, and Peacock, was able to transmit the tumors with filtrates. Because McIntosh was studying the virus of Rous sarcoma at the same time, Peacock thought that this virus might have spread among the birds that were used in the study of chemically induced tumors.

From the Department of Pathology, Cornell University Medical College.

This study has been aided by grants from the Anna Fuller Fund, the Jane Coffin Childs Memorial Fund, the John and Mary R. Markle Foundation and the Ophthalmological Foundation, Inc.

1. Rous, P.: J. Exper. Med. **12**:696, 1910.
2. Rous, P.: J. Exper. Med. **13**:397, 1911.
3. Rous, P., and Murphy, J. B.: J. A. M. A. **56**:741, 1911.
4. Murphy, J. B., and Landsteiner, K.: J. Exper. Med. **41**:807, 1925.
5. Peacock, P. R.: J. Path. & Bact. **36**:141, 1933.
6. McIntosh, J.: Brit. J. Exper. Path. **14**:422, 1933.

McIntosh also described leukemia in the birds that received tar. From his data it is not possible to determine whether the diseases observed in the blood-forming organs were due to leukemia or a leukemoid reaction, were produced by the agent of sarcoma, occurred independently or were a secondary reaction to sarcoma. Mellanby [7] inoculated chickens with Rous sarcoma and dibenzanthracene-induced tumor at the same time. The filtrate of the latter gave rise to a Rous sarcoma, whereas the cellular material produced a growth similar to the original chemical tumor with none of the characteristics of the Rous sarcoma. This experiment indicates that in fowl the filtrable agent of Rous sarcoma can pass into a chemically induced tumor which itself has no filtrable agent.

In view of the confusion existing in this field, it seemed desirable to reinvestigate the problem, and find out, if possible, whether tumors induced by a chemical contain a filtrable virus. In the experiments which will now be described, we attempted to propagate fowl tumors induced by benzpyrene in chicken eyes and breasts and on the chorioallantoic membranes of chick embryos. Tumors which were highly malignant to their hosts were produced in chickens with benzpyrene. However, they could be passed to only a few of the many birds that were inoculated and in this respect differed from the readily transmissible virus tumors.

Since attempts to propagate these benzpyrene-induced tumors by inoculation into the anterior chambers of chicken eyes and on the chorioallantoic membranes of chick embryos were unsuccessful, it was necessary to control the experiment by propagating one of our readily transmissible sarcomas by the same procedures.

MATERIAL AND METHODS

Young Rhode Island Red and Barred Plymouth Rock chickens weighing about 500 Gm. were used in the transmission experiments.

The method used by us for implanting material on the chorioallantois of the chick embryo has been described in detail by Burnet.[8] Fertile eggs from Rhode Island Red hens were incubated for ten days at 37.5 C. and turned daily. They were then candled to locate the air sac and the embryo, which were outlined with a wax pencil. By the use of an electric rotary tool with a carborundum disk attached to the shaft, a triangular area, 1 cm. to a side, was cut through the shell over the outlined embryo without penetrating the adherent underlying shell membrane. The upper half of the egg was cleansed with 70 per cent alcohol, and a thin layer of melted paraffin was placed over the triangular cut. A small

7. Mellanby, E.: J. Path. & Bact. **46**:447, 1938.

8. Burnet, F. M.: The Use of the Developing Egg in Virus Research, Medical Research Council, Special Report Series, no. 220, London, His Majesty's Stationery Office, 1936.

opening 2 to 3 mm. in size was made over the air sac with the rotary tool and likewise coated with paraffin. The triangle of shell was lifted off, and the air sac punctured with a sharp instrument. By creating suction with a rubber tube opening into the air sac, the chorioallantois was separated from the shell membrane, which was then removed under sterile precautions and the inoculum placed over the chorioallantois. The triangle was rimmed with petrolatum and a cover slip placed over it. The egg was returned to the incubator for a period of ten days. Then the upper part of the shell was removed, and the chorioallantois collected in Tyrode's solution. Part of the membrane was placed in Zenker's fixative, and part was cut into 1 mm. particles for further passage. The collected material was always cultured prior to further implantation on egg membranes.

The following technic was used for the transplantation of tumor particles into the anterior chambers of chicken eyes: The eye was first anesthetized with 2 per cent butyn solution, and the eyelids were separated with a wire speculum. A 3 mm. incision was made at the corneoscleral junction with an angle keratome. The tumor implant was grasped with a curved iris forceps and was inserted through the incision into the anterior chamber. The tumor particle was observed at frequent intervals in order to determine the increase in size.

PRODUCTION OF BENZPYRENE TUMORS IN CHICKENS

Six chickens were each given 2 intramuscular injections of 10 and 5 mg. of benzpyrene in lard at the same site at an interval of twenty-eight days. Two months later 3 of these chickens had palpable tumors at the sites of inoculation. A fourth chicken died after three months without having had a detectable tumor during life, but at autopsy a tumor measuring 3 by 2 by 2 cm. was found, which proved to be fibrosarcoma. Transmission of this growth was not attempted. The 2 remaining chickens, killed ten months after inoculation, had no tumor.

The chickens with benzpyrene-induced tumors that were studied experimentally will be designated as chickens 1, 2 and 3. Biopsy specimens were removed from these tumor-bearing chickens, and part of each tissue specimen removed was fixed for microscopic study. Another part was set up in tissue cultures, while the remaining part was dried and frozen.

Chicken 1.—A biopsy was made of a tumor measuring 2 cm. in the greatest diameter eighty-five days after the first injection. A section of this tumor showed fibrosarcoma (fig. 1 *A*). The chicken died six months after the initial injection of benzpyrene. At autopsy 2 tumors were found. One was in the breast and the other in the thigh. Each measured about 4 cm. in the greatest diameter. These tumors were firm, cut with increased resistance and showed a tendency toward nodular formation. Soft yellow areas that measured from 2 to 4 mm. across were noted in the central parts. The liver was found to be moderately enlarged and contained irregularly scattered discrete gray-white nodules measuring 2 mm. in diameter. Microscopically the portal spaces showed

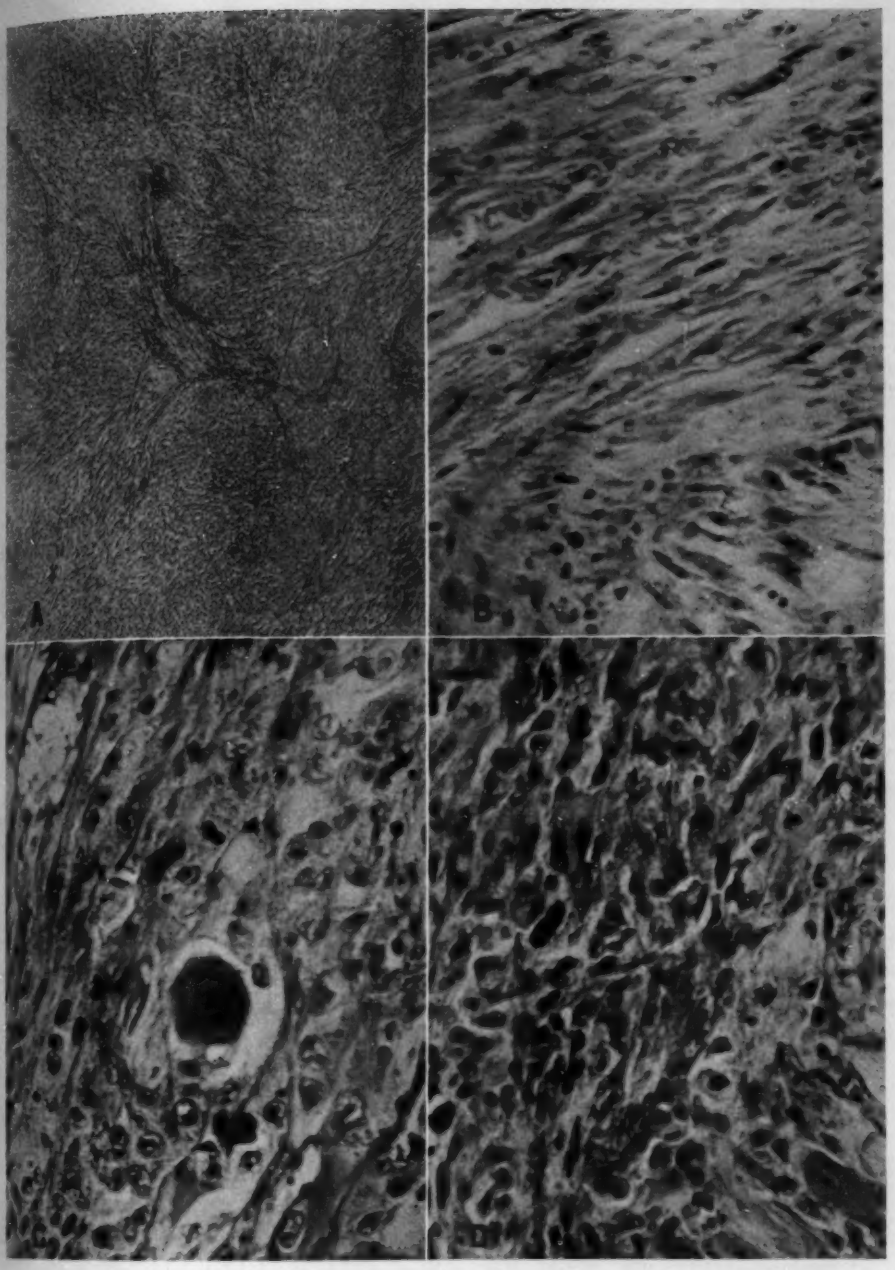


Fig. 1.—*A*, low power photomicrograph of the benzpyrene tumor from chicken 1 to illustrate the general arrangement of the tumor cells in whorls; × 39. *B*, photomicrograph of the tumor from chicken 2; × 357. The tumor cells are elongated, vary in size and resemble fibroblasts. Many nuclei show chromatolysis. *C*, photomicrograph of the tumor from chicken 3; × 357. The cells are large and irregular in size and shape. The nuclei are either vesicular or pyknotic. Few mitotic figures are seen. There is a large multinucleated giant cell in the field. *D*, section from the transplanted tumor of chicken 3 a; × 357. The tumor cells are irregular in shape, size and arrangement, and the nuclei are pyknotic and vary in size. A few muscle fibers are seen.

extensive infiltrations of myelocytes. The spleen was twice the normal size and mottled with many round white areas, sections of which showed masses of myelocytes. There were many similar cells in the splenic pulp and capillaries. This chicken had myelocytic leukemia associated with spindle cell sarcoma.

Chicken 2.—A biopsy was performed three months after injection of benzpyrene on a tumor measuring 3 by 2 by 2.5 cm. The section of this tumor reproduced in figure 1 *B* shows sarcoma. The bird was killed nine months after the initial injection. A tumor measuring 10 by 8 by 6 cm. was found in the right breast and a smaller tumor in the left breast. These tumors were firm and cut with increased resistance; soft areas of necrosis that measured 2 to 6 mm. across were noted in the central parts. The lungs contained a few small metastatic nodules, situated beneath the pleura, measuring 5 mm. across. The liver was slightly enlarged and contained minute raised grayish nodules; micro-

TABLE 1.—*Transplantation Experiments with Benzpyrene-Induced Tumors*

	Site of Injection										
	Intramuscular Site			Anterior Chamber			Intravenous Site			Chorioallantois	
Tumor	Chicks	Injections	Chicks in Which Tumors Developed	Chicks	Injections	Chicks in Which Tumors Developed	Chicks	Injections	Chicks in Which Tumors Developed	Eggs	Eggs in Which Growth Developed
1	24	26	0	4	4	0	4	4	0	...	..
2	15	20	0	6	6	0	..	..	..	144	6
3	47	99	1	23	34	0	5	5	0	....	..

scopic examination showed these to be infiltrations of small lymphocytes. The spleen was slightly enlarged but was normal on microscopic examination.

Chicken 3.—A small tumor which was palpated at the site of injection of benzpyrene after 2 months became progressively larger and at the end of five months measured 4 cm. in the greatest diameter. At this time a biopsy was made, and a section of the growth (sarcoma) is reproduced in figure 1 *C*. This bird lived for two months after biopsy. At autopsy it was emaciated, and in the left breast there was a large necrotic tumor measuring 7 by 6 by 5 cm. There were small tumor nodules in the heart, lungs, liver and kidneys, all of which proved to be sarcoma.

Eighty-six chickens (table 1) received a total of 208 injections of fragments of the three benzpyrene-induced tumors. There were 155 intramuscular and 9 intravenous injections. As the figures indicate, many chickens received multiple injections. Only 1 (3 a) of 10 chickens receiving tumor fragments from chicken 3 showed a growth. This tumor, illustrated in figure 1 *D*, was noted forty-six days after the injec-

tion and at that time measured 1.5 cm. in its greatest diameter. A second subpassage was made into 13 chickens, and only in 1 (3 b) did small tumors develop that were recognizable at autopsy. No other passage of these tumors was attempted.

ATTEMPTS TO GROW THE BENZPYRENE-INDUCED TUMOR ON THE CHORIOALLANTOIS AND IN THE CHICKEN'S EYE

Woodruff and Goodpasture [9] showed that the chorioallantois is a favorable site for the study of virus infections. Since Rous sarcoma can be grown by introducing either a filtrate or cellular material onto the egg membrane, an attempt was made to propagate a benzpyrene-induced tumor by this method in order to determine whether a virus was associated with it. Two of the chickens with benzpyrene-induced tumors had died before we commenced work with the egg membranes, and only chicken 2 was available for these studies. The attempt to grow this tumor on the chorioallantoic membrane was unsuccessful.

Fragments of this growth were implanted on 8 egg membranes 11 days old, in one of which a nodule developed, measuring 4 by 2 by 2 mm., after ten days' incubation. However, histologic examination of this growth proved that it was a proliferation of the mesoderm and not a tumor. The cells were compact and very regular in cytologic detail; they contained few mitotic figures. They did not bear any resemblance to the cells of the original tumor. Nevertheless, small fragments of this nodule were placed on 8 more membranes, but no tumor was produced. Another subpassage was performed, and this also gave negative results. The implantation did not kill any of the developing embryos. The nodule grown on the egg membrane was inoculated into the breasts of 4 chickens, which remained free from tumors after five months.

In all, 144 inoculations on the chorioallantoic membrane were done, and none was successful (table 1).

Transplants from the tumors of chickens 1, 2 and 3 were placed in the anterior chambers of chickens' eyes in 44 instances. They did not increase in size during a period of four months, and in most cases they became smaller.

It was conceivable that the benzpyrene tumors failed to grow because the experimental birds were not inbred. In an attempt to adapt the sarcoma cells to alien plasma, material from different biopsy specimens of the benzpyrene-induced tumors was first grown in tissue cultures. A total of 26 chickens was inoculated with these cultures, none with success. Four series of specimens of the same material were carried to three successive passages on the chorioallantois but were also unsuccessful.

9. Woodruff, A. M., and Goodpasture, E. W.: Am. J. Path. **7**:209, 1931.

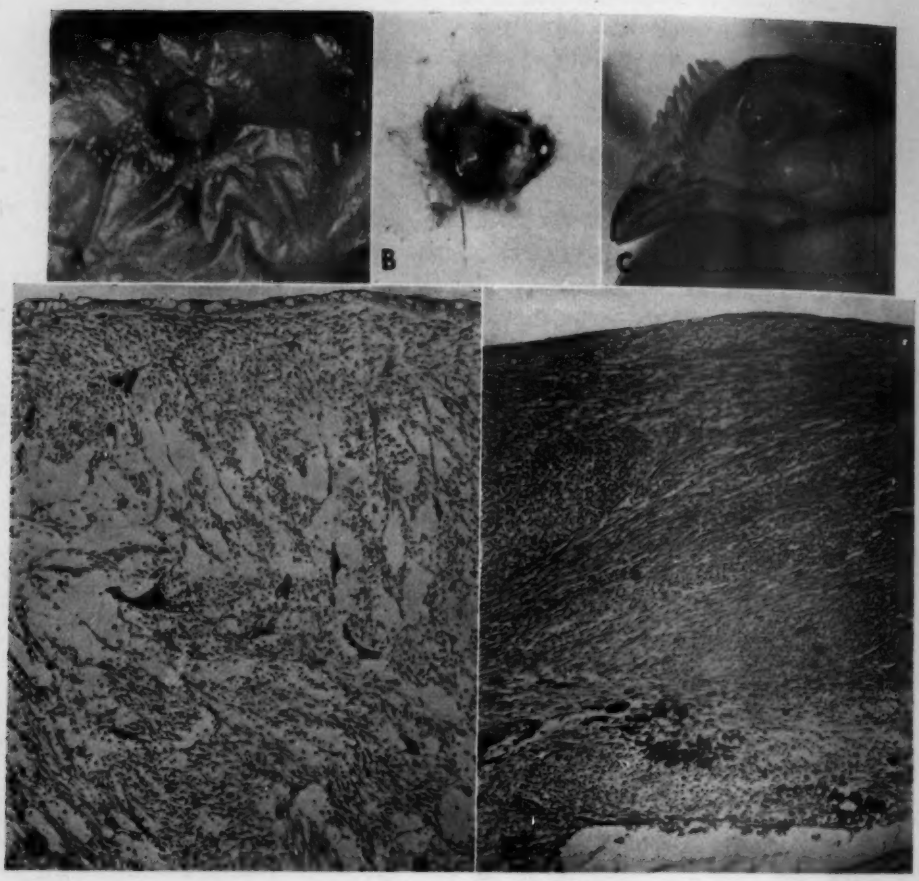


Fig. 2.—*A*, tumor measuring 11 by 9 by 8 mm., produced in ten days on the chorioallantois of the chick embryo by implantation of a fragment of sarcoma 13; × 1.03. *B*, higher magnification of the tumor illustrated in *A*. The chorioallantois has been removed, and a discrete growth is seen on the inner aspect of the membrane. *C*, photograph to show the eye of a chicken twenty-seven days after implantation of sarcoma 13 in the anterior chamber. The palpebral fissure is wide because of increased intraocular pressure. The pupil and iris are obscured by gray opaque tumor tissue. *D*, low power photomicrograph showing a layer of ectodermal cells of the chorioallantois; × 79. Some of these are degenerated. Beneath this layer and involving only the mesoderm is a diffuse infiltration of tumor cells, which are spindle shaped and loosely connected. Few mitotic figures are seen. *E*, photomicrograph showing diffuse infiltration of tumor tissue through the ciliary body and iris; × 73. Some of the spindle-shaped cells are arranged in compact manner and others are loosely bound. A considerable number of macrophages which contain pigment are scattered throughout the tissue in the region of the ciliary body.

The cells grown in the tissue cultures were like fibroblasts, but since we are unable to distinguish in vitro normal fibroblasts of chickens from malignant fibroblasts, and since inoculation experiments were unsuccessful, the character of these cells remains uncertain. Tissue cultures prepared from virus-induced tumors, however, invariably produced cells that were proved to be sarcoma by reinoculation into chickens.

PRODUCTION OF VIRUS-INDUCED TUMOR ON THE CHORIOALLANTOIS

Because the benzpyrene tumors failed to grow on the chorioallantois, it seemed essential to control this phase of the work with a virus tumor known to be transmissible. For this purpose, sarcoma 13 of Stubbs and Furth [10] was selected.

This virus produces sarcoma at the site of intramuscular injection with or without erythroleukosis and also stimulates endothelial cells of the blood-forming organs to neoplastic growth.

This sarcoma was readily grown on the chorioallantois in numerous subpassages. Particles of tumor that measured 1 mm. in the greatest diameter were placed on egg membranes, and after ten days' incubation each was about ten times as large as it was originally. The gross and the microscopic appearance of the sarcoma on the chorioallantois are illustrated in figure 2 *A, B* and *D*. The growth was most distinct on the inner aspect of the membrane and appeared as a gray, opaque soft nodule, measuring 11 by 9 by 8 mm. On the fifth passage it was collected from the egg and inoculated into the breast of 4 chickens, in 3 of which tumors developed. In another experiment the growth was collected after the first subpassage and inoculated into 3 chickens. At the sites of inoculation in all 3 chickens tumors developed, and, of 9 chickens that were given intramuscular injections of the sarcoma grown on the egg membranes, 8 had metastases in the liver and spleen.

These experiments indicate that cells of sarcoma 13 can be readily conveyed on the chorioallantois and that the growth retains its virulence for at least five generations. Moreover, the growth on egg membranes appeared as good in the eighth generation as in the first and the fifth.

PRODUCTION OF TUMORS WITH CELL-FREE VIRUS OF SARCOMA 13

Attempts were made in four experiments to produce tumor on the chorioallantoic membrane by means of cell-free virus. Two of these attempts were successful (table 2).

In experiment A, the tumor extract was freed from cells by centrifugation at 3,000 revolutions per minute for fifteen minutes, and the supernatant fluid was recentrifugated at 8,000 revolutions per minute for thirty minutes in an angle

10. Stubbs, E. L., and Furth, J.: J. Exper. Med. **61**:593, 1935.

centrifuge. The supernatant fluid was used for inoculation in chickens and for implantation on the chorioallantois. The material was cell free when examined under the microscope and was sterile when cultured aerobically and anaerobically.

Experiment B was carried out in a similar manner except that frozen cellular tumor material suspended in Tyrode's solution was used in the preparation of the extract. The resultant supernatant fluid was concentrated by centrifugation in a Bauer and Pickels [11] high speed centrifuge run at 28,000 revolutions per minute for forty-five minutes.

In experiments C and D, 10 Gm. of tumor (sarcoma) was finely chopped and ground with sterile sand; 250 cc. of Tyrode's solution was added and the suspension shaken for fifteen minutes. Sand and solid particles were removed by centrifugation. The supernatant fluid was passed through a Seitz E. K. filter, with a culture of Bacillus prodigiosus being used as a control. This filtrate was also free from cells.

In 12 of 32 egg membranes, each of which had received 0.25 cc. of cell-free extract, tumors were observed (table 2). Discrete, raised gray-white opaque nodules, 1 to 3 mm. in diameter, covered the inoculated areas. Fragments of membrane containing the growth were reimplanted

TABLE 2.—*Production of Sarcoma with Cell-Free Virus (Sarcoma 13)*

Experiment	Chorioallantois		Pectoral Muscle	
	Number Inoculated	Number with Tumors	Number Inoculated	Number with Tumors
A	8	6	3	3
B	8	0	2	2
C	8	6		
D	8	0	4	2
Total	32	12	9	7

on two successive series of egg membranes in each of the four experiments, and all produced growths of about the same character and size as those of the first passage. The malignant nature of these lesions was not determined by inoculation in birds. Similar lesions, however, were never produced on membranes by implantation of sterile tumor-free tissue.

These experiments indicate that the virus of sarcoma 13 readily produces tumor on the chorioallantoic membrane of the chick embryo and is capable of producing a growth in the breast of the chicken.

PROPAGATION OF SARCOMA 13 IN THE ANTERIOR CHAMBER OF THE CHICKEN'S EYE

Fragments of sarcoma 13, 1 mm. in diameter, were placed in the anterior chambers of chickens' eyes under sterile precautions, and ten

11. Bauer, J. H., and Pickels, E. G.: J. Exper. Med. **64**:503, 1936.

days after the transplantation an average increase of twice the initial size of the inoculums was noted in one half of the birds. A tumor that occupied practically all of the anterior chamber of a chicken's eye twenty-seven days after inoculation is shown in figure 2 *C*. This tumor infiltrated the iris, ciliary body and part of the choroid, and loosely growing tumor cells also invaded the posterior chamber of the eye (fig. 2 *E*).

Table 3 shows that 22 inoculations were made into anterior chambers of chickens' eyes, and in 11 instances progressively growing tumors developed. The percentage of successful ocular implantations was 50, as compared with 65.7 in the chicken breast and 70.8 on the chorioallantois. These experiments show that the anterior chamber of the eye is a satisfactory site in which to grow a virus tumor. Although statistical proof is lacking, our observations indicate that the rate of growth is more rapid on the chorioallantois than in the breast of the young chicken or in the anterior chamber of the eye.

TABLE 3.—*Transplantation Experiments with Sarcoma 13*

Site of Injection	Injections	Tumors	Percentage with Tumors
Anterior chamber	22	11	50
Intramuscular site	38	25	65.7
Chorioallantois	120	85	70.8

COMMENT

Numerous birds which had been given injections of different viruses of leukosis and sarcoma were in the same animal room with chickens that were being given injections of benzpyrene. The fact that the benzpyrine-induced tumors could be passed with the greatest difficulty in chickens and not at all on the chorioallantois indicates that they do not contain any of the viruses used in other studies in this laboratory. Since we have failed to propagate the benzpyrene tumors in series, the question arises whether these growths were malignant. Microscopically they were indistinguishable from the chicken tumors that were produced by virus and from the sarcoma growths in mice. They were locally invasive, and metastases were found in 2 of the 3 chickens which received benzpyrene. Malignancy and transplantability obviously do not go hand in hand.

The success of other workers with various tumor implantations on the chorioallantoic membrane and in the anterior chamber of the eye led us to investigate whether the benzpyrene tumors could be propagated by these methods. These experiments were unsuccessful, but control experiments with the readily transplantable chicken sarcoma showed that the anterior chamber of the eye is slightly less favorable for the

propagation of sarcoma than the breast of the chicken. The chorioallantois, however, appears to be as good, if not better, than the breast as a site for the propagation of the growth.

Greene and Saxton [12] transplanted rabbit uterine adenoma into anterior chambers of rabbits' eyes. When tumor fragments were used, 50 per cent grew successfully. Although we worked with a virus-induced tumor of fowl, our percentage of transplants that grew is similar.

While our figures show that the anterior chamber of the eye is less favorable for the propagation of a tumor than the other two sites mentioned, the percentage of transplants showing growth is sufficiently high to demonstrate the value of this procedure. The main advantage of the method is that one may make repeated and direct observations of the tumor.

Attempts were made in a series of experiments to grow malignant lymphoid cells of mice on the chorioallantois. These experiments have so far given negative results but are still in progress.

SUMMARY AND CONCLUSIONS

Sarcoma was produced in 3 chickens by injection of benzpyrene. Attempts to transplant 2 of the tumors into 39 chickens were unsuccessful, and the third tumor could be transplanted in but 1 of the 47 chickens in which it was inoculated. In subpassage this tumor grew in only 1 of 10 chickens into which it was passed, and on further passage, in 1 of 13.

These tumors could not be propagated in the anterior chambers of chickens' eyes, and attempts to propagate 1 of the 3 tumors on the chorioallantoic membrane of the chick embryo were unsuccessful.

The fact that the benzpyrene-induced tumors were transplanted with such difficulty indicates that if they contained any virus they did not contain any of the readily transmissible viruses studied in this laboratory.

The virus-induced sarcoma 13 studied in control experiments was grown on the chorioallantois in 70.8 per cent, in the breast of the chickens in 65.7 per cent and in the anterior chamber of the chicken's eye in 50 per cent of the trials. The fact that the growth may be observed directly in the anterior chamber makes this a valuable site for the study of tumor propagation.

This tumor was transmitted on egg membranes for eight successive generations.

Cell-free material from sarcoma 13 produced sarcoma on the chorioallantoic membrane of the chick embryo as well as in the breast of the inoculated chicken.

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12. Greene, H. S. N., and Saxton, J. A., Jr.: J. Exper. Med. **67**:691, 1938.

PRIMARY CARCINOMA OF THE LIVER

J. LOESCH, M.D.
ONEONTA, N. Y.

In the majority of cases primary carcinoma of the liver is rather easily identified and classified from the distribution and characteristic gross appearance, especially when it is in the early or moderately advanced stage, or, if the nature of the lesion is more obscure, by a careful search of the other organs to eliminate a primary tumor in those structures. However, it is frequently difficult, sometimes even impossible, to classify primary carcinoma of the liver as to histogenesis.

My co-workers and I on several occasions saw so much variation of the histologic picture during routine study of such cases or even within the same case that we were prompted to subject our autopsy material to a careful scrutiny, taking several blocks from different areas of the tumor and thereby attempting to evolve certain histologic criteria which could be used for classification in atypical cases.

According to the literature, all investigators seem to have agreed on the comparatively rare occurrence of this type of neoplasm. Thus Goldzieher and von Bókay [1] reported 18 cases in 6,000 autopsies, Rowen and Mallory [2] 9 in 6,500, Clawson and Cabot [3] 5 in 5,100, Torland [4] 10 in 6,000 and Fried [5] 4 in 1,200.

Our material comprised 11 cases of primary carcinoma of the liver in advanced stages and 3 of the neoplasm in early stages in 3,000 autopsies. The latter (cases 2, 3 and 5) were found incidentally.

REPORT OF CASES

CASE 1.—The patient was an emaciated white man, 55 years old.

The liver was cirrhotic; its weight was 6½ pounds (2,948 Gm.); contiguous to the inferior vena cava was a large necrotic tumor mass with central hemorrhage;

From the Department of Pathology, Buffalo City Hospital, and the Laboratory of the Homer Folks Tuberculosis Hospital.

1. Goldzieher, M. A., and von Bókay, Z.: Virchows Arch. f. path. Anat. **203**: 75, 1911.

2. Rowen, H. S., and Mallory, F. B.: Am. J. Path. **1**:677, 1925.

3. Clawson, B. J., and Cabot, V. S.: J. A. M. A. **80**:909, 1923.

4. Torland, cited by Herxheimer, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 1, p. 823.

5. Fried, B. M.: Am. J. M. Sc. **168**:241, 1924.

whitish nodules, ranging in size from that of a small pea to that of a large cherry, were distributed throughout the remaining portion of the organ.

Microscopically, the tumor resolved itself into acini with delicate vascularization between these structures. The acinal cells were much smaller than the liver cells and had basophilic cytoplasm. The majority of the acini contained bile in their lumens. Single tumor acini were frequently seen in the periportal lymph spaces and in larger veins, intermingled with blood. Occasionally, some of the acinal cells showed mitotic figures with formation of giant cells. Contiguous to the tumor, the hepatic parenchyma was compressed, resulting in marked degenerative changes in the liver cells.

Case 2.—The patient was a well developed, somewhat obese white man, 62 years old.

The liver was characterized by advanced cirrhosis and showed numerous large regenerative adenomatous nodules scattered throughout; in the upper half of the midportion, between the right and the left lobe, there was an encapsulated olive-sized nodule, largely friable and necrotic.

Microscopically, the structure of the nodule was like that in case 1 except that no bile was found. Serial sections revealed capsular invasion and extension along the lymph and blood spaces, with tumor cell nests a considerable distance from the main nodule. One of the tumor foci had penetrated the wall of a small vessel and therewith gave rise to further spread of the tumor.

Case 3.—The patient was a poorly nourished white man, 60 years of age.

The liver was cirrhotic; in the center of the right lobe was a tumor node, 2 cm. in diameter and encapsulated by a thick layer of connective tissue. On sections through various levels it was found that the tumor had penetrated the capsule at several points and that thrombi extended into the branches of the portal vein.

Microscopically, the cirrhotic liver presented small deposits of iron pigment in the periportal connective tissue. The structure of the tumor was like that in case 2.

Case 4.—The patient was a fairly well nourished white man, aged 62 years.

The liver was discolored diffusely greenish; it was cirrhotic, with a deep cleft along the coronary ligament; in the upper portion of the right lobe there was a large striated and lobulated tumor mass; immediately adjacent to the latter there were numerous small nodules, which compressed but did not invade the inferior vena cava or hepatic veins. Metastases were found in several thoracic vertebrae; in two situations they protruded into the spinal canal, resulting in compression of the spinal cord.

Microscopically, this tumor greatly resembled that in case 3. In addition, however, the cells were at times arranged in strings with spaces between them, suggesting hepatic sinusoids, as they were lined by slender cells resembling Kupffer cells. There were also areas where the tumor formed wide cords, with the cells varying considerably in size and shape, many of them containing acidophilic granules in the cytoplasm. Bile was nowhere demonstrable. The structure of the vertebral metastases was the same as that of the main tumor. Sometimes the variation seemed to be even greater.

Case 5.—The patient was a well nourished white man, 50 years old.

The liver was slightly enlarged, with sharp edges and a reddish brown cut surface. At the porta hepatis there was a circumscribed whitish growth, 1.75 cm.

in diameter, which had broken into a large vein, filling the entire lumen with a tumor thrombus.

Microscopically, the liver was that of a patient with acute leukemia. The hepatic cells contained a great deal of iron pigment, more pronouncedly at the periphery of each lobule. The tumor nodule, subdivided by several septums, had penetrated at one point the inner capsular layer and was spreading in a circular manner along the lymphatic spaces. It was immature and showed in the main a medullary arrangement, forming wide cords with fine vascularization between them. In a few areas, however, typical acini were seen. The lymph spaces were markedly distended, giving the growth an angiomatous character. Bile formation was nowhere demonstrable.

Case 6.—The patient was a well nourished white man, 62 years old.

The liver was cirrhotic; the parenchyma between the markedly thickened portal framework was replaced by whitish nodules of varying size, except for a small area in the left lobe. Most of the intrahepatic vascular spaces were invaded by tumor. Through a vein of the liver this growth extended into the inferior vena cava and was adherent to the wall of the vessel for 8 cm. After penetration of the wall at one point, it spread along the lumbar portion of the spine, invading simultaneously some of the intervertebral disks and the bodies of the second and third lumbar vertebrae. Small metastases were demonstrable in the first, second and third ribs and the clavicle on the left side; one small tumor nodule was found in the body of the sternum, and numerous ones, varying in size, were scattered throughout both lungs.

Histologically, the tumor proliferations resembled in the main those in case 2. In addition, in some situations the acini contained in their lumens granular acidophilic masses. The accumulation of these reached, especially in the central portions, such an extent that the lumens were distended and the surrounding cells flattened. Thus a picture not unlike that of a thyroid was produced. In other places the growth consisted of columns, the cells of which resembled hepatic cells. In some vessels (fig. 1) the blood was frequently intermingled with tumor acini, thus accounting for the wide spread of metastases. The thrombus in the inferior vena cava and the nodules in the lungs presented the same picture as the tumor in the liver. In the bone metastases, however, a more solid arrangement predominated. The cells were pleomorphic, and their cytoplasm was distended by mucoid material, which displaced the nuclei peripherally.

Case 7.—The patient was a well nourished white man, aged 60 years.

The liver was small and cirrhotic, with ill defined whitish tumor nodules from 0.5 to 2 cm. in diameter scattered throughout the organ. There were multiple metastases in the peritoneum.

Microscopically, the markedly cirrhotic liver showed small deposits of pigment in the periportal tissue. The tumor revealed great variation histologically. Thus, in one area there were mature adenomatous formations (fig. 2*A*), many of which contained inspissated bile in their lumens and were subdivided by septums and surrounded by a connective tissue capsule. Occasionally, the latter was missing and the transition from tumor to liver columns was direct. In other areas the liver parenchyma was replaced by narrow cords of tumor cells, which stained dark blue with hematoxylin and eosin (fig. 2*B*). The stroma was diffusely infiltrated by polymorphonuclear leukocytes. In other situations, again, broad columnar-like formations were noted, consisting of cells with the nuclei placed peripherally and

the cytoplasm a diffuse pink (fig. 3 *A*). Finally, areas of pleomorphic elongated tumor cells were encountered (fig. 3 *B*). Arranged in groups or single and enmeshed in young connective tissue, they simulated a sarcoma-like structure.

CASE 8.—The patient was a poorly nourished white man, 58 years old.

The liver was cirrhotic, with whitish nodules from 0.5 to 4 cm. in diameter, the largest concentration of which was in the right lobe. A tumor thrombus was found in the portal vein, beginning at the fusion of the mesenteric tributaries and extending into several intrahepatic branches. In the lower lobe of the right lung there was a metastasis 1 cm. in diameter.

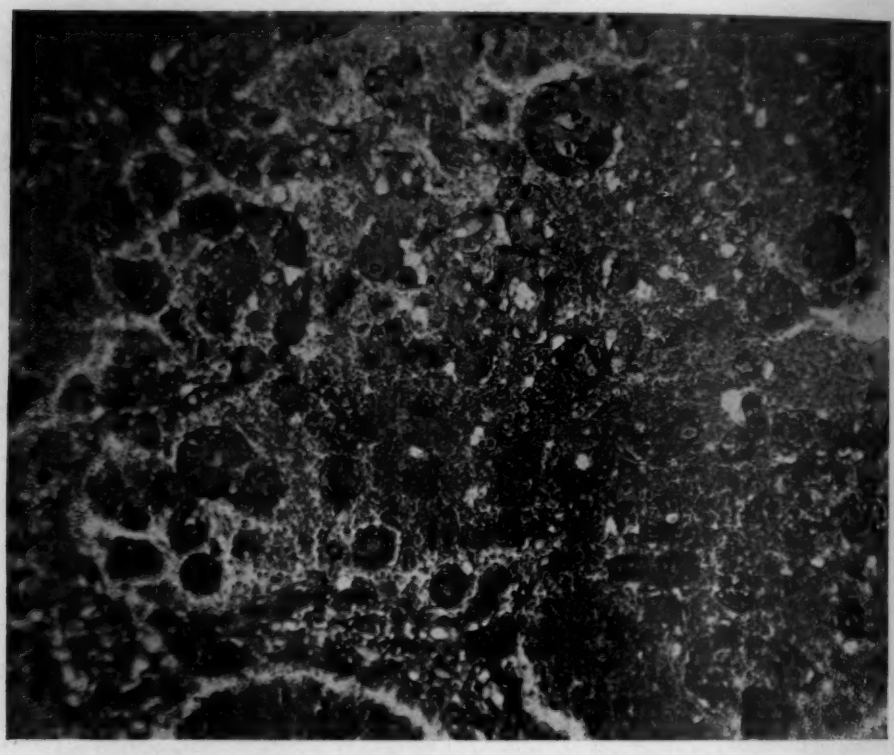


Fig. 1.—Tumor acini in a blood vessel.

Histologically, the tumor consisted chiefly of cords of cells, varying in form and size, with the cytoplasm slightly acidophilic and the nuclei strongly basophilic. Large multinucleated cells, so-called tumor giant cells, were found not only in the neighborhood of necrotic areas but also in the intact tumor. Frequently they formed true tumor columns (fig. 4). Some of the tumor cells contained in their cytoplasm granular débris and numerous polymorphonuclear leukocytes. In other sections, again, the tumor consisted of large uninucleated cells with fat vacuoles in the cytoplasm. The stroma was scant, and through it ran a fine capillary network. Thus, the picture was not unlike that of hypernephroma. Further, in other sections a pleomorphic cellular arrangement was seen as observed in some of the previous cases.

CASE 9.—The patient was a white man aged 71, fairly well nourished.

The liver was markedly enlarged, with numerous nodes protruding from its surface. Nearly the entire lower portion of the right lobe was replaced by a tumor mass, 15 cm. in diameter and necrotic in the center. There were several tumor proliferations in the remaining portions of the liver. A small metastasis was detected in the lower lobe of the left lung and in a lymph gland at the bifurcation of the trachea.

Microscopically, the liver revealed foci of necrosis and a central zone of congestion with atrophy of the liver cells. Peripherally there were small deposits of fat. The periportal connective tissue was but slightly increased. The tumor was arranged in cords. Some of the cellular elements resembled hyalinized liver cells; the rest were pleomorphic. The latter predominated, varied in size and had no similarity to any cell type. Some stained very dark; others were light. Along the edges of the tumor a gradual shading off into normal tissue was noted. Also, tumor giant cells and tumor invasion of vessels were frequently seen. The extrahepatic metastases revealed the picture of the primary tumor.

CASE 10.—The patient was a white man aged 60 years, fairly well nourished.

The liver was markedly enlarged; its weight was 6 pounds (2721.5 Gm.). Numerous whitish nodes, varying in size, were scattered throughout the organ; a tumor thrombus was found in the portal vein and metastases in the portal lymph nodes.

The tumor consisted of cells that were cylindric or cuboid; in their cytoplasm they contained acidophilic granules; they were arranged either in single layers with a delicate stroma between them or in solid thick cords. In the latter, central necrosis was sometimes observed. Also, this tumor frequently showed multinucleated tumor giant cells. The metastases in the portal lymph nodes presented great similarity to the picture just described. The medullary arrangement, however, predominated.

CASE 11.—The patient was a white man aged 60 years, poorly nourished.

The liver was cirrhotic, with large adenomatous nodes as evidence of marked regeneration. Almost the entire right lobe was replaced by a tumor mass that was necrotic in the center. The left lobe contained a few small nodes. In the portal vein a tumor thrombus filled the entire lumen and extended into several intrahepatic branches.

The tumor was of a medullary type throughout. On longitudinal sections the cells formed broad columns, and between the latter was a delicate vascular network. Thus this growth resembled somewhat that in the previous case. Tumor giant cells were seen in almost every field. The liver cell cords adjacent to the tumor nodes were compressed, indicating expansive rather than infiltrative growth. The periportal tissue contained tumor in nearly every blood and lymph space. The tumor formations in the lungs were like those described in the liver.

CASE 12.—The patient was a white woman aged 45 years, well nourished.

On exploratory laparotomy the diagnosis of carcinoma of the liver was made, and a piece of tissue was removed. The histologic diagnosis was carcinoma with cells resembling liver cells. Permission for an autopsy was refused.

CASE 13.—The patient was a white man of 76 years, poorly nourished.

In the right lobe of the cirrhotic liver there was a large tumor mass, with numerous tumor nodules of varying size in the remaining portions of the liver. There were metastases in two portal lymph nodes.

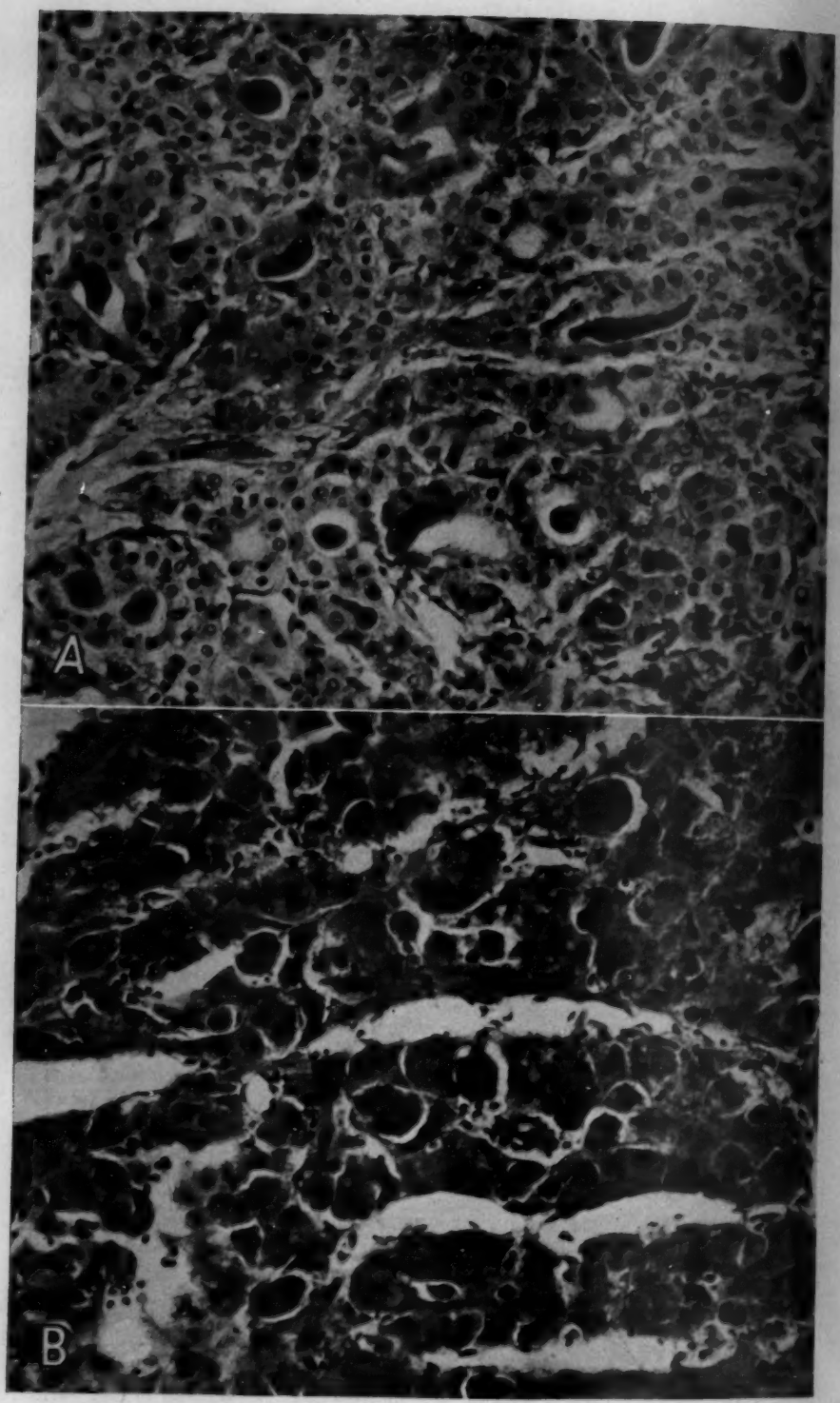


Fig. 2.—Variations of the histologic picture in case 7 (see text).

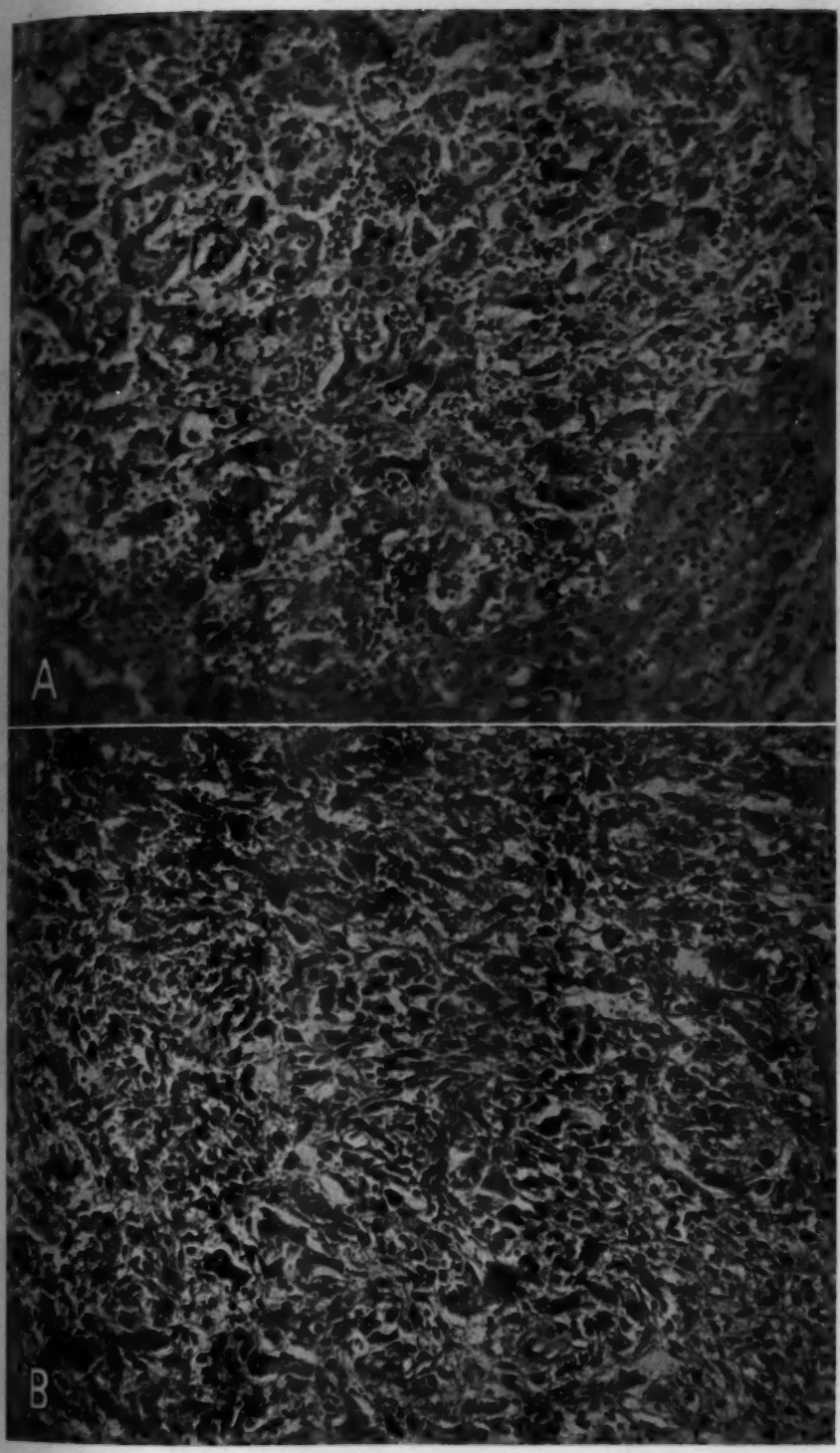


Fig. 3.—Further variations of the histologic picture in case 7.

CASE 14.—The patient was a very obese white woman, aged about 50 years.

The liver was enlarged, and the surface was finely granular. On section the entire organ was studded with tumor nodes, the greatest concentration of which was in the lower portion of the right lobe.

As the last two tumors had about the same histologic picture they will be described together. The growth had a tubular arrangement consisting of one layer of cells which were at times somewhat cuboid and at other times cylindric, resembling the lining cells of bile ducts. Bile was nowhere found; also there were no tumor giant cells. Between the tubular structures, which were more

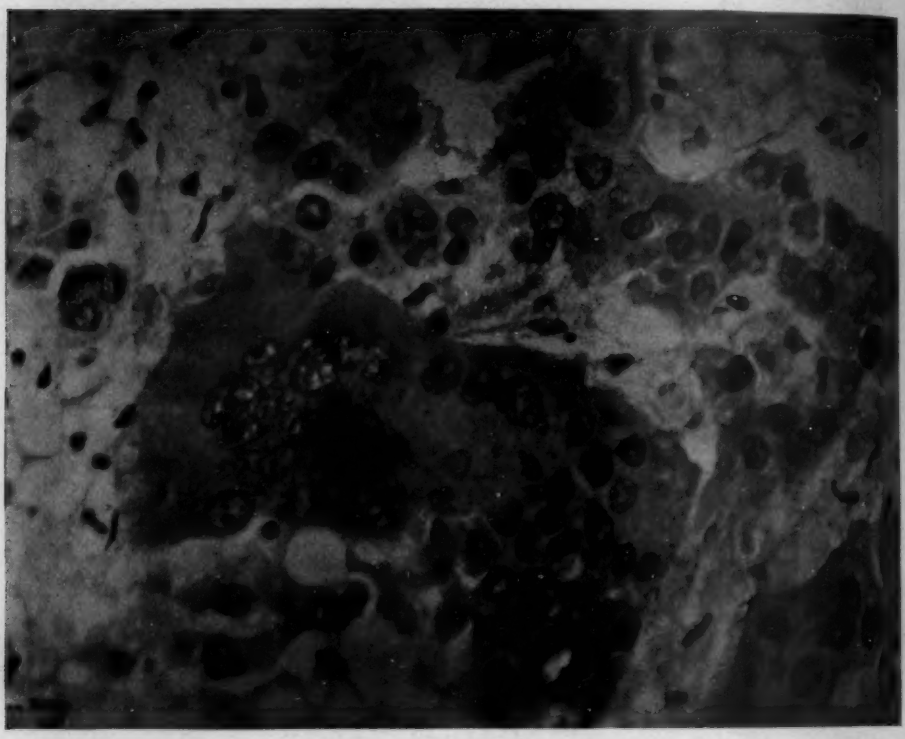


Fig. 4.—A tumor giant cell forming a tumor column.

clearly defined in the peripheral portions of the nodes, there were broad strands of connective tissue. These were diffusely infiltrated with polymorphonuclear leukocytes and carried but scanty vascularization. The metastases in the lymph nodes had merely an imitation of the tubular structure found in the liver, as the arrangement of the cells was much more dense.

COMMENT

On a careful analysis, giving attention to cell structure and to arrangement of cells, to the presence of stroma and to its width, to the degree of vascularization and to the functional capacity of the cells,

cases 1 to 12 were classified as cases of liver cell and cases 13 to 14 as cases of bile duct cell carcinoma. The terms "hepatoma" and "cholangioma," introduced by Yamagiwa [6] and used in more recent articles by Fried [5] and Orsos,[7] were omitted because they might lead to confusion inasmuch as they do not express the malignant character of the tumor and therefore might be justifiably used for the benign adenoma. If, however, such designations are used for the malignant tumors, then the attribute "carcinomatosum" or "malignum" should be added.

The liver cell type is unique. Acini formations, which characterize the more mature tumor, were found in cases 1, 2, 3, 4, 5 and 7. These contained inspissated bile in cases 1 and 7 only. Mucoid droplets in the cellular cytoplasm, resulting in collections of this material in the lumens of acini, were found in cases 4, 6, 7 and 9. In case 6 the lumens were distended in situations, the lining cells being flattened and a structure not unlike thyroid produced. This formation of mucus was not regarded as a degenerative process but as a part of the functional activity of the carcinoma cell. This belief was supported by the absence of disintegrative material in these areas. The fat deposits in cases 4 and 6 were other evidences of the functional capacity of the cancer cells. In case 5 another feature was noted, which consisted of a marked distention of the vascular network, imitating an angiomatous type of tumor. Case 7 was characterized by rosette formation with inspissated bile in the center and by columns of carcinoma cells that were distinctly basophilic. The stroma which was infiltrated by polymorphonuclear leukocytes was wide. In some areas there were columns of cells containing a mucoid material that took a violet stain with hematoxylin and eosin, and in others, cellular foci, suggesting a sarcomatous tumor. This case illustrated very clearly the fallacy of expressing any conclusive opinion as to the diagnosis based on the morphologic structure of the tumor in any one single area. My colleagues and I have been profoundly impressed by this fact in the study of this series of cases. Such terms as "sarçoma" and "carcinosarcoma" would probably have been excluded in some of the reported cases if sections from many areas had been studied. In case 8 the hypernephroma-like structure and the pleomorphism of the cells were outstanding features.

Multinucleated tumor giant cells were found in cases 1, 6, 8, 10 and 11, varying in number and size. Occasionally they formed an entire tumor column. They are, in my opinion, peculiarly characteristic of carcinoma of the liver cell type and were missed in bile duct cell carcinoma. Etiologically considered, these cells are probably in some of

6. Yamagiwa, K.: Virchows Arch. f. path. Anat. **206**:437, 1911.
7. Orsos, F.: Beitr. z. path. Anat. u. z. allg. Path. **84**:33, 1930.

the cases the result of precipitated division of the nuclei without division of the cytoplasm. This seems to be the only explanation when regressive changes are not in evidence. In the majority of cases, however, I regard them, in agreement with Petersen,[7a] as a sign of slowly occurring cellular disintegration. This disintegration is evidenced by the granular débris in the cytoplasm and the immigration of numerous leukocytes therein. The nucleus, however, still divides, but not the cytoplasm. The stroma in nearly all these cases was delicate, carrying a fine vascular network.

The bile duct cell type showed a tubular arrangement, a uniform histologic picture, a broad stroma, no formation of bile, and tumor giant cells nowhere.

A considerable literature has accumulated dealing with the question as to whether primary carcinoma of the liver is multicentric or unicentric in origin. Adherents of the first theory were Travis,[8] Löhlein,[9] Yamagiwa,[6] Goldzieher and von Bokáy,[1] Counsellor and McIndoe[10] and others. The chief representative of the second theory was Ribbert.[11] He claimed also that the spread of the tumor takes place by contiguity and along the distributing lines of the portal vein. In this statement he was supported by Herxheimer[12] and Saltikow, who pointed to the frequent invasion of smaller vessels, and was followed by Wegelin,[13] Lissauer,[14] Winternitz,[15] Karsner[16] and others. Goldzieher and von Bokáy strengthened the first theory by not having found in serial sections direct continuity from one growth to another. This is also true for my case 2. In the latter, as well as in cases 3, 5 and 13, in all of which the early stages of carcinoma were represented, the unicentric origin and mode of propagation were illustrated clearly, as seen from the descriptions. Theoretically, the possibility of multicentric origin has to be allowed in some cirrhotic livers, since the regenerative hyperplastic nodes are usually multiple, and the latter are closely related to the development of carcinoma. Yet it is my belief that it occurs very seldom, and but rarely will there be an occasion affording a definite proof. Thus, in case 6, which is one of diffuse liver cell car-

7a. Petersen, W.: Beitr. z. klin. Chir. **34**:682, 1902.

8. Travis, C. H.: Bull. Johns Hopkins Hosp. **13**:108, 1902.

9. Löhlein, W.: Beitr. z. path. Anat. u. z. allg. Path. **42**:531, 1907.

10. Counsellor, V. S., and McIndoe, A. H.: Am. J. Path. **2**:557, 1926.

11. Ribbert, M. W. H.: Das Karzinom des Menschen, sein Bau, sein Wachstum, seine Entstehung, Bonn, F. Cohen, 1911.

12. Herxheimer, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 1, p. 868.

13. Wegelin, K.: Virchows Arch. f. path. Anat. **179**:95, 1905.

14. Lissauer, M.: Virchows Arch. f. path. Anat. **202**:57, 1910.

15. Winternitz, M. C.: Johns Hopkins Hosp. Rep. **17**:143, 1916.

16. Karsner, H. T.: Arch. Int. Med. **8**:238, 1911.

cinoma, a multicentric origin could readily be considered, as many tumor nodules were of the same size. But this probably was due to the resistance of the thick portal framework, from which they could be pulled out with ease. The diffuse involvement, on the other hand, can be explained without any hesitation on the basis of the great number of carcinomatous acini found intermingled with the blood of the portal vein.

Intrahepatic metastatic dissemination occurred through the small blood vessels, lymph spaces and lymph vessels. As the latter were invaded along the large vessels, a spread, accompanying the portal rami-

Vascular Invasion and Extrahepatic Metastases of Primary Carcinoma of the Liver

Case	Branches of Hepatic Vein	Inferior Vena Cava	Branches of Portal Vein	Portal Lymph Nodes	Peritoneum	Skeleton	Lungs	Bronchial Lymph Nodes
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	+	0	0	0	0	0
4	0	0	0	0	0	Thoracic part of spine	0	0
5	0	0	0	0	0	0	0	0
6	+	+	+	0	0	Left 1st, 2d, 3d rib and clavicle; body of sternum; 2d and 3d lumbar vertebrae	Bilateral	0
7	0	0	0	0	+	0	0	0
8	0	0	+	0	0	0	Lower lobe of right lung	0
9	0	0	0	+	0	0	Lower lobe of left lung	+
10	0	0	+	+	0	0	0	0
11	0	0	+	0	0	0	Bilateral	0
12	0	0	0	0	0	0	0	0
13	0	0	+	+	0	0	0	0
14	0	0	0	0	0	0	0	0

fications, resulted. Tumor thrombi growing into the branches of the portal vein against the blood stream and extending even into the main trunk were another source of propagation. They were observed in 6 of these cases.

One instance was noted in which a tumor thrombus extended from a hepatic vein into the inferior vena cava and penetrated the wall of the latter at one point.

In order to get a clear view of the extrahepatic metastatic distribution the table may be referred to. It seems strange that metastases could be demonstrated in the regional lymph nodes in 3 cases only. The peritoneum was involved once, the lungs four times, a bronchial lymph node once and the skeleton twice; in a case in which the skeleton was

involved epidural extension from the thoracic portion of the spine resulted in compression of the spinal cord.

Many of the investigators of the subject called attention to the fact that cirrhosis was present in a majority of cases (Eggel, cited by Herxheimer [12]; Rowen and Mallory [2]) or in all of them (Strong and Pitts [17]; Karsner [16]; Winternitz [15]). It was found in 12 of the 14 cases reported here. Wegelin [13] and Löhlein [9] believed the cirrhosis to be secondary to the carcinoma, as a reaction to irritation due to the presence of the tumor. At present, however, all investigators agree that cirrhosis is the primary condition and a predisposing factor. One must, however, recognize that slight proliferation of connective tissue takes place around the tumor, as was pointed out by Counsellor and McIndoe,[10] Winternitz[15] and others. But this never leads to a picture of diffuse cirrhosis. An example in the present series was seen in case 5, in which a distinct connective tissue reaction was noted in the immediate vicinity of the tumor but was not demonstrable in the uninvolved parts of the liver.

It is a well established fact that destruction of parenchyma is followed by regeneration to a varying degree. In the more pronounced lesions the latter seems to follow with greater ease. Then loss of control of growth, at one time or another, results in the formation of a tumor.

Comparing the 12 cases reported here with 94 cases of atrophic cirrhosis, covering the same period, I arrived at the conclusion that on the average primary carcinoma of the liver developed in 1 of every 8 cases of the latter number. Pigmentary cirrhosis, which Mallory found frequently associated with carcinoma of the liver, was of no etiologic significance in the present series; but slight pigment deposits were occasionally seen in the periportal connective tissue, as in cases 4 and 7. Those in the liver cells in case 5 were unquestionably due to destruction of red cells during the course of the acute leukemia. Since adenomatous hyperplasia was absent in this case, the tumor originated presumably in an embryonic rest or in a clump of liver cells which were separated from the surrounding parenchyma by connective tissue, the regulatory control being lost at a later period. The truth in either case cannot be determined with any degree of certainty. This case, then, may be grouped with those reported by Hedinger, Helvestine, Wiest and others, since primary carcinoma of the liver without cirrhosis is generally found in young persons.

SUMMARY

Fourteen cases of primary carcinoma of the liver are reported, 12 of the liver cell and 2 of the cholangiocellular type. The hepatocellular type is unique in the variation of the microscopic picture, not only in

17. Strong, G. F., and Pitts, H. H.: Arch. Int. Med. **46**:105, 1930.

different cases but also frequently in the same case; the cholangiocellular type is of a uniform structure, with an adenocarcinomatous picture.

Metastases within the liver were demonstrated in the early stage of the development of the primary tumor. The intrahepatic spread of the growth took place along the portal vessels, lymph spaces and smaller blood vessels and from tumor thrombi of the proximal branches and the main trunk of the portal vein. In case 6 a tumor thrombus extended along a hepatic vein far into the vena cava inferior, perforating the wall of the latter at one point.

Extrahepatic metastases were not uncommon; their distribution is seen in the table.

Carcinoma of the liver is unicentric in origin, as was clearly demonstrated in the study of the early cases. The same explanation is also compatible with diffuse involvement, as seen from the descriptions given in foregoing sections.

In 3,000 autopsies 94 cases of atrophic cirrhosis were found, in 12 of which primary carcinoma was demonstrated; 2 additional cases of primary carcinoma of the liver without cirrhosis were demonstrated, the apparent origin being an embryonic rest or a clump of cells separated from the mother tissue.

It was found twelves times in males and twice in females. The average age of the patients was 59.5 years.

CLASSIFICATION AND PATHOLOGY OF RENAL DISEASE IN THE DOG

COMPARISON WITH NEPHRITIS IN MAN

FRANK BLOOM, D.V.M.
FLUSHING, L. I., N. Y.

The problem of Bright's disease in man has been the subject of intensive experimentation and research. Numerous investigators have utilized the dog in the artificial production of nephropathies. On the whole, it is true that the renal lesions induced have little resemblance either to the glomerulonephritis which occurs in man or to the renal inflammatory disease which the dog suffers naturally. It is desirable to be familiar with this spontaneous renal disease of the dog in order to interpret better any experimentally produced nephropathy. Furthermore, renal disease occurs in this animal with comparatively great frequency, and if this fact is not recognized, incorrect conclusions may be drawn from the results of any experimental investigations in which the kidneys are examined.

The principal renal inflammatory disease of the dog is true interstitial nephritis, which may be acute, subacute or chronic. When acute, it closely resembles in many respects the acute interstitial nephritis in man which occasionally occurs as a complication of scarlet fever and other acute infections. The so-called chronic interstitial nephritis in man must not be confused with the true chronic interstitial nephritis of the dog. In the latter, the process has its inception in the interstitial tissue, and the tubular and glomerular changes are purely secondary. Aschoff's [1] statement that in some cases contracted kidney in man may commence from acute interstitial nephritis, while not generally believed, is further controverted by the fact that true chronic interstitial nephritis in the dog is easily differentiated from contracted kidney of man resulting from glomerulonephritis or arterial disease. It is thus an interesting fact of comparative pathology that interstitial nephritis which is of very minor importance in man should be of such great significance in the dog, while glomerulonephritis is of no importance in this animal but is the dominant renal inflammatory disease of man.

1. Aschoff, K. A. L.: Pathologische Anatomie, ed. 6, Jena, Gustav Fischer, 1923.

In a perusal of the medical literature it is noted that the fact that the dog suffers principally from interstitial nephritis is generally not recognized and that many descriptions are given of the spontaneous renal disease of this animal which do not closely correspond to that which actually occurs. Veterinary textbooks and writers with few exceptions also give an erroneous concept of nephritis in the dog. The terms used and the descriptions seem to have been borrowed from the older medical textbooks.

LITERATURE

The older veterinary pathologists were fully aware that the dogs frequently showed renal lesions at postmortem examination. In most cases the description of the lesions was based purely on macroscopic examination, and it remained for Davis [2] to be first to describe interstitial nephritis in the dog. Joest [3] and Nieberle and Cohrs [4] stressed the frequent occurrence of interstitial nephritis in dogs. Henschen [5] gave a good survey of the older literature. Müller,[6] Brumley,[7] Hutyra, Marek and Manninger [8] and Jakob,[9] in their descriptions of canine nephritis, placed exceedingly little stress on interstitial nephritis.

Workers on experimental nephritis also have stressed the great frequency of spontaneous renal disease in dogs, though in most instances either the author did not state what type was present or the pathologic description is faulty. Microscopically Dayton [10] found 1 of 21 dogs with normal kidneys. However, he described the histologic changes as glomerulonephritis and, in the more chronic cases, sclerosis of the arteries. Interstitial changes, such as fibrosis and round cell infiltrations, were also mentioned but no recognition was given that these were primary. MacNider [11] in a series of 237 dogs found 42

2. Davis, U.: Die histologischen Veränderungen bei der Nephritis des Hundes, Dissert., Berne, E. Hess, 1908.

3. Joest, E.: Spezielle pathologische Anatomie der Haustiere, Berlin, R. Schoetz, 1924, vol. 3.

4. Nieberle, K., and Cohrs, P.: Lehrbuch der speziellen pathologische Anatomie der Haustiere, Jena, Gustav Fischer, 1931.

5. Henschen, F., in Joest.[3]

6. Müller, G. A.: Diseases of the Dog and Their Treatment, ed. 5, translated by A. Glass, Chicago, A. Eger, 1926.

7. Brumley, O.: Diseases of the Small Domestic Animals, Philadelphia, Lea & Febiger, 1938.

8. Hutyra, F.; Marek, J., and Manninger, R.: Special Pathology and Therapeutics of the Diseases of Domestic Animals, translated by C. F. Marshall and C. M. Ottley and edited by J. R. Greig, J. R. Mohler and A. Eichhorn, London, Baillière, Tindall & Cox, 1938.

9. Jakob, H.: Innere Krankheiten des Hundes, Stuttgart, Ferdinand Enke. 1924.

10. Dayton, H.: J. M. Research **31**:177, 1914.

11. MacNider, W. deB.: J. M. Research **34**:177, 1916.

nephropathic. He described the renal lesions as primary glomerulonephritis with absence of acute inflammatory glomerular changes. He made no mention of interstitial round cell infiltrations. To term such lesions glomerulonephritis is certainly contradictory to the type of renal lesions the dog commonly suffers from. Winternitz and Quinby [12] described spontaneous nephritis in a dog which closely resembled interstitial nephritis. However, they stated further that while spontaneous renal lesions are not uncommon in dogs occasionally extensive anatomic changes occur that are similar, even in their detail, to those found in the progressive nonsuppurative types of nephritis in man. Hartman, Bolliger and Doub [13] in a study of roentgen ray nephritis in dogs stated that one of the reasons that dogs were selected for their experiments is that the animals are less notorious for incidence of spontaneous nephritis. The evidence is to the contrary, renal lesions being more frequently found than is commonly believed.

Since this paper was written, Whipple and Robscheit-Robbins[13a] have stated that 11 per cent of the dogs in their anemia colony had glomerulonephritis at autopsy. Undoubtedly these animals had interstitial nephritis, and it would indeed be remarkable if glomerulonephritis were found in so many dogs.

MATERIAL AND METHODS

This report is based on observations on a series of 4,123 dogs of all ages and breeds that were brought to the hospital for examination and treatment. Of this number, 274 suffered from clinical renal disease, a diagnosis confirmed by laboratory examinations of the blood and urine, and were brought to the hospital expressly because of the uremic symptoms. Of these, 70 died or were killed because the condition was deemed incurable. In another group of 200 animals that died of various causes and in which uremic symptoms were absent, histologic examinations of the kidneys showed lesions of focal interstitial nephritis in 108 dogs.

Necropsies were made immediately and the tissues fixed in a 10 per cent dilution of neutral solution of formaldehyde U. S. P., Zenker's fluid, Maximow's fluid and Flemming's solution and were stained with hematoxylin and eosin, the Lee-Brown modification of Mallory's aniline blue,[13b] Wright's stain, Gram's stain, iron-hematoxylin, Van Gieson's stain, Weigert's stain and Wilder's silver stain for reticulum.[13b]

GENERAL OBSERVATIONS

The clinical features of the disease and the laboratory studies of the blood and urine have been described elsewhere.[14] The symptoms

12. Winternitz, M. C., and Quinby, W. C.: J. Urol. **1**:139, 1917.

13. Hartman, F. W.; Bolliger, A., and Doub, H. P.: J. A. M. A. **88**:139, 1927.

13a. Whipple, G. H., and Robscheit-Robbins, F. S.: J. Exper. Med. **69**:485, 1939.

13b. Lee, B.: The Microtomist's Vade-Mecum, Philadelphia, P. Blakiston's Son & Co., 1937.

14. Bloom, F.: J. Am. Vet. M. A. **44**:679, 1937; Cornell Vet. **27**:130, 1937; Lederle Vet. Bull., March-April and May-June, 1938, vol. 7.

in general are those of uremia. Edema and hematuria are exceedingly rare. Cardiac hypertrophy is usually absent, and ophthalmoscopic examination of the fundi reveals no vascular changes. Convulsions do not occur, though muscular twitchings are sometimes present. The etiologic factors are unknown, though a large variety of causes have been implicated. The disease occurs more frequently in male animals, and practically all dogs over 8 years of age evidence some degree of interstitial nephritis.

CLASSIFICATION

The following classification is proposed, based on the histologic examinations:

I. Noninflammatory nephropathies
   1. Nephrosis
      (*a*) Mild, moderate and severe (necrotizing)
      (*b*) Amyloid

II. Inflammatory nephropathies
   1. Suppurative nephritis
   2. Interstitial nephritis
      (*a*) Acute, subacute and chronic

Glomerulonephritis and arteriosclerotic kidneys have not been encountered in this group of animals. One kidney showed spotty calcification of the larger arteries, involving principally the media and closely resembling Mönckeberg's sclerosis. Lipoid nephrosis is unknown in the dog. In the group of 70 animals which died of uremia, histologic examination of the kidneys showed the following percental incidence of the different nephropathies: necrotizing nephrosis, 2.8; amyloid nephrosis, 1.4; suppurative nephritis, 7.1, and interstitial nephritis, 88.7.

PATHOLOGY

Nephrosis and suppurative nephritis are similar in their histologic aspects to the same conditions in man and therefore do not require special description.

The following discussion therefore is a discussion of interstitial nephritis. Macroscopically, the kidneys show a variety of appearances depending on the type of disease present. The acute focal type is characterized by the presence in the cortex and, if the condition is of the severer type, in the outer zone of the medulla of small pinpoint to pinhead sized areas of a whitish gray color on the surface. The acute diffuse type shows these areas increased in size and in number. If the nephritis is of a mild and focal type, the macroscopic appearance may be entirely normal. The capsule peels easily, and there is rarely any

bulging on section. The cortical markings are usually distinct but they may be obscured if the infiltrations are dense and extensive. The color of the kidney is usually normal, though occasionally it is a mottled deeper red. In healed focal nephritis, the kidney appears normal with the exception of occasional pale gray scars. The number of scars depends on the original severity of the disease.

The subacute form offers a variety of appearances and in some cases is difficult to distinguish from the chronic form. Generally, the number of scars is increased, and this produces an irregular nodular surface. The capsule is usually adherent to the scarred areas, and the organ is firmer on palpation. Irregularly scattered, particularly in the cortex, are small grayish areas. The striations are obscured in the areas of infiltration and scarring.

In the chronic form, the kidney is shrunken and contracted. It is fibrous and firm and cuts with difficulty. The capsule is commonly thickened and adherent, though this is not an absolute rule; it may strip easily. The kidney is practically always a uniform pale tan color. The surface is nodular, though the size of the nodules varies considerably in different kidneys. The cortex is irregularly decreased in size, and the markings are usually obscured and obliterated. Frequently there are present smaller and larger cystlike structures in the medulla and occasionally in the cortex.

Microscopically, the picture is also variable but in general consists of the true inflammatory changes of infiltration and proliferation. The infiltration is principally that of the interstitial tissue by mononuclear cells, and the proliferation is chiefly that of fibroblastic formation of connective tissue. The infiltrating cells have the appearance of lymphocytes, though some closely resemble plasma cells. The nucleus is oval and may be situated in the central or in the peripheral portion of the cell. In fresh infiltrations these cells are large; the nuclei contain less chromatin and therefore stain lighter. In old infiltrations the cells are smaller, the chromatin material of the nuclei is more compact, and the nuclei stain very deeply and appear pyknotic. The cytoplasm contains no granules, and on vital staining with trypan blue these cells do not take up the stain. In some cases neutrophils are present in the infiltrations, though the predominant cells are the lymphocytes. The infiltrations may be perivascular, periglomerular and intertubular in distribution.

The number of infiltrative cells depends on the acuteness and extent of the nephritis. In the focal type the infiltrations vary from several to large numbers of lymphocytes scattered irregularly throughout the kidney. Most of the cellular collections are located in the cortex and the outer stripe of the outer zone of the medulla. In the acute diffuse type the infiltrations are particularly dense and heavy. The tubules in the

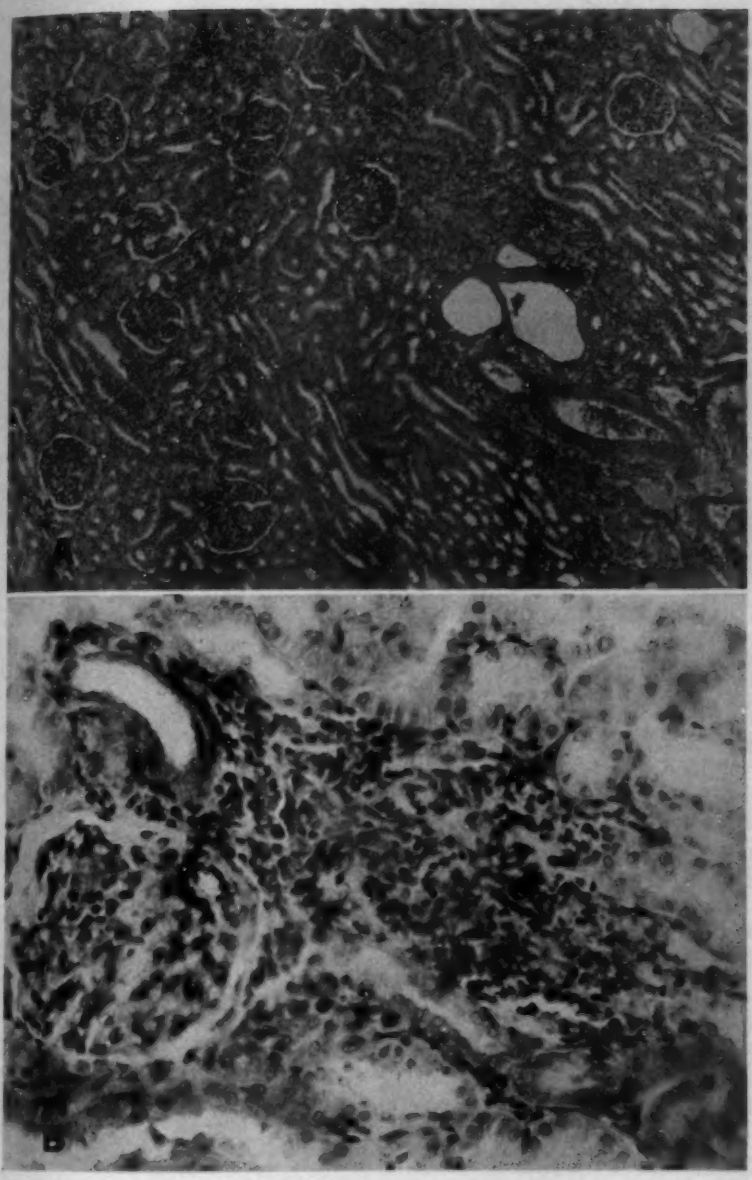


Fig. 1.—*A*, acute focal interstitial nephritis; hematoxylin and eosin; × 60. Note scattered collections of lymphocytic cells in focal areas. The glomeruli and tubules are essentially normal. *B*, greater magnification of the lymphocytic infiltration of *A*; Wright's stain; × 280.

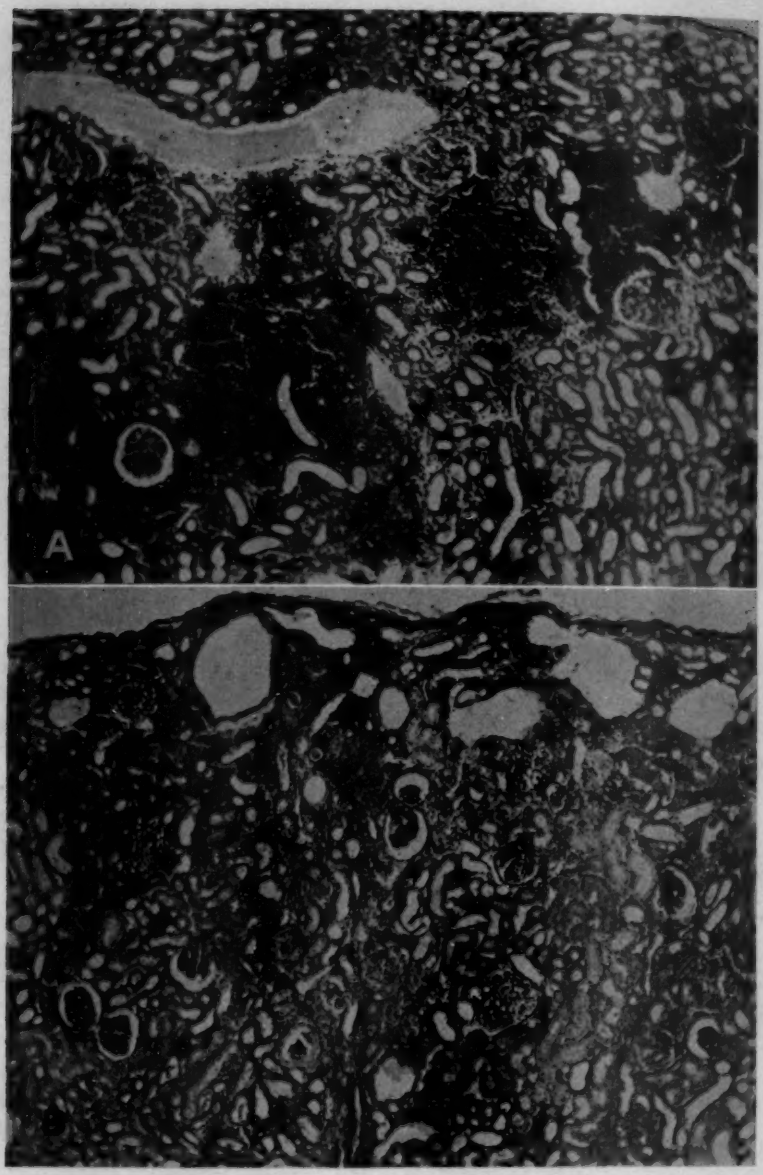


Fig. 2.—*A*, acute diffuse interstitial nephritis; hematoxylin and eosin; × 60. Note very heavy collections of lymphocytic cells diffusely distributed throughout the organ. The glomeruli even though included in the areas of lymphocytic infiltration are normal. The tubules in the heavily infiltrated areas have disappeared. *B*, focal scar extending from the outermost portion of the cortex to the medulla; hematoxylin and eosin; × 60. Several of the included glomeruli are fibrosed. Outside the scar the renal parenchyma appears normal.

infiltrated areas may be displaced or destroyed; usually the epithelial nuclei persist in moderate numbers. The reticulum in these areas is usually sparse. In general the infiltrations are lighter in the inner zone of the medulla and rarely occur as large groups of cells.

The glomeruli and blood vessels show practically no changes with the possible exception of mild congestion in some cases, even though heavily surrounded by inflammatory cells. The tubules involved evidence degenerative changes of different degrees, though many may be normal. In the focal type the tubular changes are of a mild nature, consisting of cloudy swelling and slight vacuolation of the cytoplasm. In the diffuse type the parenchymatous degeneration may be severe, with necrosis of the cells and disappearance of their nuclei. The degenerative changes are most prominent in the proximal convoluted tubules. Hyaline casts are commonly found and occasionally are of a granular nature with nuclear remnants. In the cortex, particularly, are frequently present regenerated epithelial cells, which contain large oval nuclei having a vesicular structure and boundaries that are indistinct or absent. Many times mitotic figures are present.

The aforementioned changes can terminate in several ways: 1. They may disappear completely by resolution and absorption. 2. They may continually progress until more of the renal structure is involved. 3. They may heal with the production of connective tissue.

The formation of scars is frequently met with in routine histologic examination of dogs' kidneys. They arise in the following manner: Fibroblasts appear in the areas of lymphocytic infiltration, with the formation of fibrous connective tissue. As the fibrosis increases, there is a corresponding reduction in the number of round cells, though they rarely disappear entirely. With the Van Gieson and the Weigert stain, the connective tissue is of the fibrous type and is richly cellular in the earlier stages of the process. The tubules and glomeruli in the scarred areas are also altered; the tubules may disappear or persist as scars or they may become dilated, showing a thin atrophic epithelium, and frequently they contain hyaline casts; the glomeruli may be distorted, fibrosed or hyalinized and may show well advanced periglomerular fibrosis.

In some cases fresh lymphocytic infiltrations appear, and the process of infiltration and scarring appears to be continuous. This constitutes the subacute form. In such kidneys the pathologic alterations are decidedly more advanced than in the acute type. The fibrosis is increased, and there are larger numbers of affected glomeruli and distorted and dilated tubules. Throughout the kidney are fresh lymphocytic infiltrations with the corresponding tubular changes. However, a large amount of the renal tissue is normal and free from alterations.

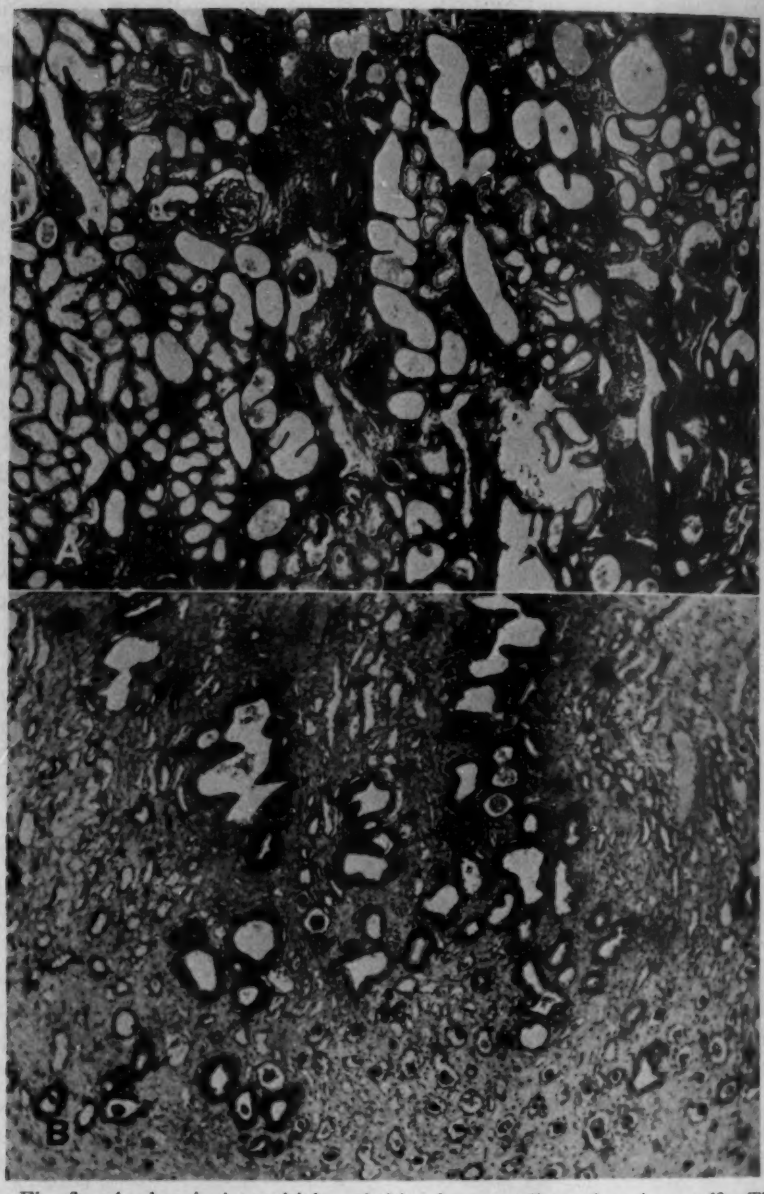


Fig. 3.—*A*, chronic interstitial nephritis; hematoxylin and eosin; × 60. The cortex shows diffuse proliferation of interstitial connective tissue with scattered lymphocytic infiltrations. The tubules show varying distortions, from collapse to dilatation. The epithelium of the dilated tubules has been reduced to a thin, atrophic layer. Some of the glomeruli are fibrosed, while others are distorted. *B*, chronic interstitial nephritis; hematoxylin and eosin; × 60. Section of medulla showing the distended collecting tubules with hyperplastic epithelium. There is diffuse proliferation of intertubular connective tissue.

Eventually the subacute form may result in chronic interstitial nephritis. In the latter, the quantitative structural changes vary considerably. The connective tissue proliferation is extensive in all cases, though in some the cortex is more affected, and in others the medulla is more involved. Large areas of the organ may be converted into masses of fibrous collagen. Scattered throughout the fibrosis are collections of lymphocytic cells with no definite distribution. The glomerular changes are similar to those found in the focal scars but are more extensive. However, in many kidneys the majority of the glomeruli show little change. The tubular changes vary considerably. In clinically compensated conditions the cortex may appear almost normal, though the medulla is extensively affected. The number of dilated tubules is greatly increased. This dilatation is exceedingly irregular, and the epithelium is very flattened. The convoluted tubules that persist in their apparent normal structural basis show decided parenchymatous degeneration, and their lumens contain granular material. Fairly constant in the inner zone of the medulla is the presence of dilated hyperplastic collecting tubules that have a glandlike appearance. The cell boundaries of such tubules are indistinct, and the nuclei are arranged in irregular rows. In the cortex and outer stripe of the outer zone of the medulla are occasionally present hypertrophied tubules with tall columnar epithelium. In some cases calcareous deposits are present in the fibrotic pericapsular tissue and around the atrophied tubules. In all cases the arteries are normal and show no intimal changes even though surrounded by inflammatory tissue.

SUMMARY

In contrast to what is observed in man, in the dog, interstitial nephritis is the most common renal inflammatory disease and may be acute, subacute and chronic. The initial pathologic process consists of lymphocytic infiltrations in the interstitial tissue and is perivascular, periglomerular and intertubular in distribution. Such infiltrations may be replaced partially or completely by fibrous connective tissue. In some cases fresh lymphocytic infiltrations appear, with further production of fibrosis, so that eventually the entire kidney may be involved. In such kidneys there are extensive fibrosis, secondary glomerular change and dilatation of tubules, showing thin, atrophic epithelium. In addition, some tubules may be hypertrophied and others hyperplastic.

Glomerulonephritis and arteriosclerotic kidneys practically never occur in the dog.

137-138 Northern Boulevard.

Case Reports

CERVICAL POLYPS IN BOTH OF PRESUMABLY IDENTICAL TWINS

JEAN GOWING, M.D., PHILADELPHIA, AND MADGE THURLOW MACKLIN, M.D., LL.D., LONDON, ONTARIO, CANADA

Tumors occurring in twins are of great interest, both when the twins are of the one ovum and when they are of the two ovum type, and also when one twin only and when both twins are affected. All such cases should be reported. For that reason we are putting on record a case of cervical polyps in twins who we think are probably monozygous because of the following points of resemblance: (1) They were indistinguishable before puberty. Later, one twin gained more in weight than the other, thus altering their similarity to some degree. (2) Both now have hazel eyes with (3) marked arcus senilis; (4) the hair in both shows the same amount of graying; (5) the skin in both has the same sallow tint; (6) both have arthritis of the knees; (7) both have arteriosclerosis; (8) both react similarly to the test of taste for phenylthiocarbamide, both agreeing that it is practically tasteless, only "a little bitter."

Mrs. C. B., married and with children (her husband was a drunkard), was first seen at the age of 59. She had had a prolapsed uterus for thirteen years. Her menopause began at the age of 49. She had slight bleeding for six months before she came for examination, which revealed perineal lacerations and a cervical polyp. The lacerations were repaired, and the polyp was removed. She had been well, without any return of the polypous growth, for seven years.

The twin sister of Mrs. C. B., Mrs. L. S., married and with children (her husband also was a drunkard), was examined at the age of 64, at which time she weighed 65 pounds (29.5 Kg.) more than her sister. She had had slight irregular bleeding for four years and feared she had cancer. Examination showed a moderate cystocele and a rectocele, and also a cervical polyp, which was removed. It was considerably larger than her sister's had been, which was to be expected inasmuch as the sister's had been operated on six months after the onset of symptoms, and hers, not until four years after the onset.

Four years later a second polyp developed, which was removed. For the pathologist's report which follows, we are indebted to Dr. Helen Ingleby, of the Woman's Medical College of Pennsylvania. "The polyp contains dilated glands embedded in cellular connective tissue. In some places the stroma is packed with inflammatory cells and the epithelium is undergoing metaplasia toward the squamous type. The cells are large and swollen, and, although they have multiplied, they appear to be degenerating and not neoplastic. There is no evidence of malignant change. The diagnosis is: cervical polyp showing severe inflammation."

The patient also has a lump developing in her breast, for which she has been treated by roentgen rays, her physician preferring this type of therapy because of her very high blood pressure. Hence no pathologic diagnosis of the variety of this tumor of the breast is available, there having been no biopsy. The first twin, Mrs. C. B., has as yet no signs of any lump in her breast.

Cervical polyp and prolapse of the uterus are apparently not rare conditions. Dr. Catharine MacFarlane, of Philadelphia, has given us the following data: On a gynecologic service there of 400 women a year, 2.5 per cent, or 10, a year exhibit prolapse of the uterus. Cervical polyp is encountered in about the same number, namely, 2.5 per cent. The true incidence of the latter is higher, however, being observed in about 6 per cent of gynecologic patients, as some patients are treated in physicians' examining rooms, without admission to a hospital. Inasmuch as not all women are gynecologic patients, these figures do not give the incidence of these conditions in women in general. The presence of two such conditions as cervical polyp and lax vaginal walls in two sisters with presumably the same inheritance would be expected if each condition had a definite genetic basis but would be found relatively seldom in two sisters if the two conditions were dependent wholly on extraneous factors. Both probably have a genetic basis, modifiable perhaps to greater or lesser degree by extrinsic causes.

There has been no other report in the literature, as far as we have been able to ascertain, of polypoid growth on the cervix in twins. One case of prolapse of the uterus, grade 3, and cystocele, grade 2, in both of identical twin sisters has been reported by Hines and Piper [1] of the Mayo Clinic.

SUMMARY

Cervical polyps, benign in character, were found in twin sisters who were presumably of the one ovum type. They occurred at approximately the same age in both patients. Both had lax vaginal walls, resulting in prolapsed uterus in one twin and in cystocele and rectocele in the other. In one twin a tumor has developed in the breast; there is as yet no sign of tumor of the breast in the second twin.

1. Hines, E. A., Jr., and Piper, M. C.: Proc. Staff Meet., Mayo Clin. **12**:815, 1937.

FETAL PERICARDITIS

R. W. Kelley, M.D., St. Louis

That fetal pericarditis is rare is shown by a review of the rather extensive literature regarding fetal infections. Instances of fetal endocarditis are common, and the possible association with the production of congenital heart disease has been frequently discussed. Only 1 case of fetal pericarditis has been found recorded in the literature. This was reported by Job, Lévy and Morlot.[1]

In this instance the infant was born at term, after a normal pregnancy. The labor was normal. Only after great difficulty were respirations initiated, and the infant remained cyanotic till death, which occurred four hours after delivery. At autopsy the pericardial sac contained a serofibrinous exudate and the epicardial surface of the heart showed small opaque plaques. The subpericardial tissues were edematous. In addition, there was acute fibrinous endocarditis, with verrucous vegetations on the mitral and tricuspid valves. The Wassermann reaction of the mother's blood was negative. There is no report of cultures taken or of microscopic studies. This infant had large areas of desquamation on the palms and soles, which led the authors to suspect syphilis as the etiologic agent.

The case to be reported here is the first instance of fetal pericarditis in approximately 7,000 autopsies performed by this department.

REPORT OF A CASE

A 33 year old colored woman, married, was first seen in October, 1924. At that time the Wassermann reaction was positive, and she received one course of treatment with a preparation of bismuth and with arsenicals. In August 1935 she was given a "rest," after which she did not return for further antisyphilitic treatment. All Wassermann and Kahn tests subsequent to the first one, including those made on May 27, 1935, were negative. On Sept. 17, 1935, she sought treatment for a profuse vaginal discharge, the result of chronic endocervicitis, which had been present for two or three years. She was treated by potassium permanganate douches and given pyridium by mouth. On May 20, 1936, a diagnosis of pregnancy was made, the expected date of confinement being December 20. On May 27 a gonococcic complement fixation test was returned as being 4 plus. No cultures of the cervical discharge were made. In July the patient complained of profuse vaginal discharge and of considerable pain on urination. On August 30 at the St. Louis Maternity Hospital she was delivered, by full breech presentation, of a stillborn girl. The puerperium was reported as being uneventful. The placenta was reported as premature. No mention of placentitis was made.

The father of the stillborn infant was treated in September 1930 and again in October 1935 for gonorrhea. He gave a history on these admissions of repeated attacks of anterior urethritis over a number of years.

From the Department of Pathology, Washington University School of Medicine.

1. Job, Lévy and Morlot: Bull. Soc. d'obst. et de gynéc. **16**:171, 1927.

Necropsy.—The body was that of a stillborn Negro girl. It weighed 500 Gm. and measured 27 cm. in length. The skin showed moderate maceration. There was no apparent inflammation of the conjunctivae, nor was there any other external evidence of infection. All the serous lined cavities contained dirty brown fluid, the product of early autolytic changes. The pleural and peritoneal surfaces were discolored but smooth and free from exudate. The visceral and the parietal pericardium were thickened and adherent. When these were pulled apart there was disclosed between them a sticky, adhesive exudate. The heart weighed 11 Gm. and showed no abnormalities of development. The endocardium showed no gross lesions. The lungs were unexpanded. The abdominal viscera showed no gross abnormalities.

On microscopic examination the visceral and parietal pericardial surfaces were found covered with a thick layer of granulation tissue. This tissue was densely infiltrated by cells, most of them polymorphonuclear leukocytes. Between the thickened visceral and parietal pericardium was a thick exudate. It was formed of fibrin, large numbers of polymorphonuclear leukocytes and a few red cells and lymphocytes. In the outer layers of the myocardium the small capillary vessels were dilated. There was no inflammation of the endocardium.

The only other organs showing abnormal microscopic changes were the stomach and duodenum. In the lumen of these organs there was an exudate composed of desquamated epithelial cells, mucus and numbers of polymorphonuclear leukocytes. The mucosal lining was intact. The small capillaries of the submucosa were congested, and this layer of the wall was infiltrated with lymphocytes. The subserosal connective tissue was thickened and edematous. It was infiltrated by lymphocytes and mononuclear cells. In the thickened connective tissue were numerous large, dilated lymphatics, which contained masses of polymorphonuclear leukocytes.

Gram stains (Goodpasture-MacCallum method) of sections of pericardium and stomach were made, but no bacteria could be demonstrated. Acid-fast and Levaditi stains were made, but careful search failed to reveal any acid-fast bacilli or spirochetes. The microscopic study of the tissue failed to show any evidences of syphilis. No cultures were taken.

COMMENT

This was a case of organizing purulent pericarditis occurring in a stillborn Negro girl of approximately twenty-two weeks' gestation. The only other finding of note was the thickening and inflammatory infiltration of the subperitoneal connective tissue plus the presence in the cavity of the stomach and of the duodenum of acute inflammatory cells. The etiologic agent in the production of this acute inflammation cannot be demonstrated. Of particular interest is the positive history of gonorrhea in both mother and father.

The mother had endocervicitis with a profuse vaginal discharge during her pregnancy. She had a 4 plus complement fixation reaction to the gonococcus. Bacterial organisms could not be demonstrated in the fetal tissue even in the areas of acute inflammation. The possibility of connecting the fetal inflammation with the chronic gonorrheal infection in the mother is almost inescapable. The mode of such an infection is not clear. The placenta was not reported as offering any evidence of inflammation, nor was the amniotic fluid apparently abnormal. We found, however, acute inflammatory cells in the stomach and duodenum

and thickening and inflammatory infiltration of the subperitoneal tissues. In these thickened tissues were dilated lymphatics filled with polymorphonuclear leukocytes. A possible supposition is that the amniotic fluid was infected (perhaps by direct extension through the membrane, from the cervical canal) and that this infected fluid was swallowed, the fetal infection resulting from absorption of organisms from the gastrointestinal tract. The most prominent localization of the infection was in the pericardial sac, as evidenced by the acute purulent pericarditis.

Although the question of fetal septicemia has received considerable study, little has been reported regarding gonococcic fetal infections. In reports on large series of fetal deaths one does not find the gonococcus mentioned as an etiologic agent. Slobozianu and Herscovici [2] reviewed the literature on gonococcic fetal infections and from it collected 5 cases of gonococcic arthritis in the newborn, to which they added 3 cases of their own. In most of these cases the mother had active gonorrheal arthritis during pregnancy. In several of the cases cited arthritis did not appear in the infant until several days after delivery. The authors concluded that since there was no ophthalmia or similar evidence of external infection, the arthritis in these infants was merely the result of an infection contracted during fetal life. They expressed the belief that although the mother may not give evidence of having active septicemia during pregnancy, transient phases may occur during which organisms penetrate the placenta and enter the fetal circulation.

SUMMARY

Organizing purulent pericarditis in a stillborn Negro girl of 22 weeks' gestation is reported. In view of the positive history of gonorrhea in the mother and father, it is suggested that the fetal inflammation may have been of gonococcic origin.

2. Slobozianu, H., and Herscovici, P.: Gynéc. et obst. **28**:601, 1933.

Obituaries

ALFRED STENGEL, M.D.

1868–1939

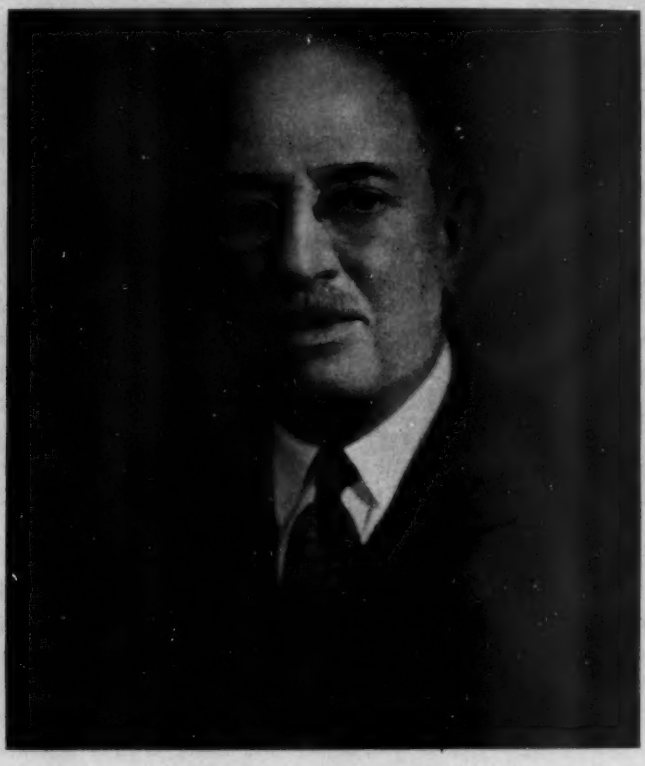
Alfred Stengel, M.D., D.Sc., LL.D. (Lafayette and Pennsylvania), died April 10, 1939, in the seventy-first year of his life.

Dr. Stengel was born in Pittsburgh on Nov. 3, 1868. He received the degree of Doctor of Medicine at the University of Pennsylvania on May 1, 1889, after which he served as intern at the Philadelphia General Hospital until Nov. 1, 1890, the last three months of the time being an extra service in the division of internal medicine. The following year he began his career at the University of Pennsylvania in the divisions of embryology and pathology and then became assistant to Dr. William Pepper and clinical clerk to him. This appointment was succeeded by that of instructor in clinical medicine, which position he held until 1899; he then became professor of clinical medicine, a grade that he held until 1911, when he became a full professor of medicine. He retired from this role in 1937, after a service of twenty-six years, to devote his major time to the duties of vice president in charge of medical affairs in the cabinet of the president of the university, Thomas S. Gates. This position, established in 1931, was the first of its kind, a part of the new system originated by Mr. Gates. During the eight years of his devotion to the duties of this office, Dr. Stengel was most active in broadening the relationships of the various schools that touch on biology and was able to associate closely the schools of medicine, veterinary medicine and biology and the Wistar Institute. The graduate school of medicine, already started, but with its greatest activity at a distance from the university, was by Dr. Stengel brought nearer physically and more closely affiliated with the splendid teaching facilities of the parent institution. In 1937 he was selected by the state of Pennsylvania to be the manager of the school of animal pathology at the university, which operates with the aforementioned divisions in collaboration with the state.

While Dr. Stengel's life was devoted in a very large part to the University of Pennsylvania, he held many other positions not directly connected with the university—positions which helped especially in the early education of the man in his chosen field. His early interest in pathology, which will be indicated by a later consideration of the literature which he produced, is shown by his close connection with the Philadelphia Pathological Society for many years, from 1891, and

the fact that in 1893 he was elected pathologist to the German Hospital, now the Lankenau, a post which he kept until 1897.

In 1895 Dr. Stengel was appointed assistant director and in 1898 full director of the William Pepper Laboratory of Clinical Medicine, a position which he retained until 1911. He was physician to the Pennsylvania Hospital from 1900 until he retired from active duty, becoming consulting physician in 1925. He was professor of medicine at the Womens' Medical College for two years, from 1896 to 1898,



ALFRED STENGEL, M.D.
1868-1939

and chief in medicine at the Howard Hospital from 1895 to 1897. He was also consulting physician to several other hospitals. He was a member of the American Philosophical Society from 1901 until his death. The Philadelphia College of Physicians elected Dr. Stengel as vice president in 1931, and from 1934 to 1936, inclusive, he served as president.

Notable, particularly in the last twenty years of his life, is the number of national committees, such as the National Research Council, on which he was invited to serve. He was president of the American

College of Physicians in 1925, 1926 and 1927, and he was on the board for the reconstruction of its constitution. He was on the board of the Wistar Institute and became its president in 1937, succeeding Effingham B. Morris. There can be added to this formidable list of services rendered by Dr. Stengel a considerable number of less conspicuous connections, but those mentioned serve to illustrate how often he was sought and how broad were his interests, how energetic his services and how capable his judgment.

As one begins to consider his professional life, one is struck by the fact that before his graduation, in 1889, and therefore before there was practically any literary activity by students, he wrote a paper on albuminuria, and during his internship at the Philadelphia General Hospital he wrote three more. The first three were for students and interns, while the last, on acute dysentery and Amoeba coli, was published in the *Medical News* of November 1890.

There is a strong suggestion, however, that Dr. Stengel had an early active interest in pediatrics, since he served, beginning in 1896, for three years as physician to the Childrens' Hospital. Some of his writings, particularly in Starr's "System of the Diseases of Children," indicate this inclination.

Dr. Stengel's interest in pathology probably was started and strengthened by his association with Juan Guiteras and Allen J. Smith. He wrote the notes for publication on Guiteras' lectures in 1891. From then on appeared a series of articles on the blood, nephritis and diseases of the heart and lungs and many reviews that indicated the pathologic analysis to which he subjected all his reading. He participated actively with Dr. William Pepper in the preparation of the latter's "System of Medicine." In 1898 he issued a "Textbook of Pathology" that ran into eight editions. In the preparation of the last three of these I had the privilege to be associated with him. In 1901 he assumed the American editorship of "Nothnagel's System of Medicine," which came out in twelve volumes. The literary contributions of Dr. Stengel ran to 179 accepted and published titles, of which only 10 were not strictly medical. Dr. Stengel was on the editorial board of the ARCHIVES OF PATHOLOGY from the start of its publication.

Dr. Stengel's development seems to have been coincident with the early changes of the influence of organic pathology in eradicating empiricism from the leading medical minds of this country. The teachings of Virchow, Cohnheim, Claude Bernard and others were reaching this country with great force. Morbid anatomy was started in this country one hundred years ago by Horner and continued in 1839 by Samuel Gross, both surgeons. It was not until 1885 that Delafield presented a separate and systematic study of pathology. However, with William Pepper, Flint and others of the American school there came a demand that pathology be thought of first in the

conception of human illness, and under this influence, in the years between his graduation and 1898, Dr. Stengel pressed himself through a discipline that led up to the publication of his book on the subject. Previous books offered cases in exemplification or discussed abstractly the possibilities of cause and effect of the morbid change. Dr. Stengel pictured the etiologic factors, the objective findings and the possible effects in a manner that forced the reader to associate the anatomic and physiologic changes with the actual clinical status of the patient—the systematic and paragraphic method practiced by the German school. This method continued to be evident throughout all his editions and indeed was shown in his teaching. He was not in speaking or writing discursive, but he followed a line of stern logical history, without dogmatism or didactics, building up a structure and then asking why and how the morbid processes were responsible and what they would mean for the future. He taught with strict objectivism, never losing the reasoning that was based on facts. He was not an abstract research worker, but he used his early knowledge of embryology and anatomy, for which he had a marvelous memory, and kept so abreast of modern physiology and chemistry that he had no difficulty in maintaining a constant balance in his dealing with a clinical problem. It is especially interesting that he could place the safest value on the details that made up the whole. He never lost sight of the woods for the trees, but a better judge of an oak never lived.

Between the early formative period and 1931, when he became vice president of the university, his major time was given to the practice of internal medicine and to his students. These men became better men because Dr. Stengel taught them to be self critical.

Personally, Dr. Stengel was of medium height or slightly less, active and definite in action, without loss of motion, of an alertness that showed itself more in his mind than in his body. Though his thought was rapid, measurement and balance were never absent. Dr. Stengel's accessibility to all who had a worthy subject was amazing. He refused no one, and his advice, based on a broad experience of human activity, was always at the disposal of the worthy visitor. His psychologic understanding seemed to extend everywhere. He employed it with amazing skill in the handling of patients, and the medical world knows that this is necessary to a great practitioner. And Dr. Stengel was a great doctor of the sick. To what degree this may have been due to his personality, he gave credit to pathology. He said once to me that he hoped no man would ever treat him for pneumonia who had never held a pneumonic lung in his hand.

The world at large, and most deeply those who knew him, grieve at the loss of a man who, although he had reached the usual promise of three score and ten, appeared to have at least a decade of joy for his friends and of value to society.

HERBERT FOX.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—The portrait of H. G. Wells painted by Wayman Adams was presented by friends of the pathologist to the University of Chicago on June 23. The portrait will hang in the lecture room of the department of pathology.

Honorary degrees have been conferred by Yale University on Hans Zinsser and E. W. Goodpasture. Dr. Zinsser has received an honorary degree from Harvard University, also.

Maurice Brodie, National Research Council fellow in medicine 1933-1934, assistant director of laboratories in Providence Hospital, Detroit, died on May 9, 1939, aged 35 years.

Anna Fuller Prize.—The first award of this prize goes to E. L. Kennaway, J. W. Cook and three other scientists at the Research Institute of the Royal Cancer Hospital of London, who will share $7,500 equally for their work on the cancerigenic hydrocarbons of coal tar.

Changes in Officers and in Board of Managers of Wistar Institute.—The Wistar Institute of Anatomy and Biology, Philadelphia, announces the election of Esmond R. Long as president of the board of managers and of Alfred N. Richards and William H. DuBarry as members of the Board. Edmond J. Farris has been appointed executive director of the institute.

Society News.—The following officers were elected at the annual meeting of the American Society of Clinical Pathologists in St. Louis: president-elect, A. V. St. George; vice president, C. L. Klenk; and secretary-treasurer, A. S. Giordano.

Finney-Howell Research Foundation.—Eight fellowships for cancer research have been renewed and six new fellowships have been awarded; also two grants-in-aid. Applications for 1940 awards must be received before January 1 next, addressed to the secretary, Dr. William A. Fisher, 1211 Cathedral Street, Baltimore.

Medical Examiner for Maryland.—In Maryland a law has been enacted, in accordance with which the coroner system will be replaced by the medical examiner system. It provides for a chief medical examiner and two assistants for the city and county of Baltimore, all on a full time, salaried basis. In each outlying county there will be a deputy examiner, whose duty it will be to make the preliminary medical investigation. If further investigation is necessary, such as postmortem or toxicologic examination, it is to be made by the medical examiner of Baltimore and the cost charged against the county for which the work is done. All appointments are to be made by a nonpaid, nonpolitical commission composed of the professor of pathology of the Johns Hopkins University, the professor of pathology of the University of Maryland, the city health commissioner of Baltimore, the state health commissioner and the state attorney general.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Viability in "Devascularized" Lymph Nodes. R. L. Holman and E. B. Self, Am. J. Path. **14**:463, 1938.

Popliteal lymph nodes of dogs when replaced in the popliteal space after complete severance of all vascular and lymphatic connections rapidly undergo massive necrosis. These nodes usually become infected and may slough out. When, however, all vascular connections are severed but one or more afferent and one or more efferent lymphatic channels remain intact, infection does not ensue, and the nodes remain viable. Chemical analyses of lymph flowing to and from these "devascularized" nodes show a sharp drop in reducing substance, bound carbon dioxide and carbon dioxide–combining power in the lymph during its passage through the node, and indicate that anaerobic glycolysis is one of the metabolic processes taking place in the viable node. These observations imply a nutritive function on the part of lymph which, so far as the authors have determined, has not been demonstrated in vivo in mammals. From Authors' Summary.

Renal Function as Affected by an Experimental Lesion of One Kidney. T. F. Nicholson, R. W. I. Urquhart and D. L. Selby, J. Exper. Med. **68**:439, 1938.

A method is described for the production of nephrosis in one kidney of an experimental animal. The normal kidney in the same animal is available as a control. The nephrosis, produced by injecting 7.5 per cent sodium tartrate, is limited to the proximal convoluted tubule. There is no histologic evidence of glomerular damage. The tubular damage results in a disturbance of the excretion of water and chloride as well as in a decrease in the clearance of urea, xylose, inulin, creatinine and phenol red, and is observed under conditions of no diuresis, sugar diuresis and salt diuresis. In the cells of the proximal tubule of the damaged kidney one finds excreted ferrocyanide. This substance is not present in the corresponding cells of the normal kidney. This observation is taken to be evidence that there has been back diffusion of ferrocyanide through the damaged cells. The decrease in clearance of creatinine and inulin, which in the dog represent glomerular filtrate, may therefore be explained by back diffusion and as not necessarily due to glomerular change. The clearance of phenol red from the damaged kidney is diminished in relation to the clearance of inulin. This is further evidence of depression in tubular activity.

From Authors' Summary.

Hypophysectomy in the Rat. A. C. Crooke and J. R. Gilmour, J. Path. & Bact. **47**:525, 1938.

A series of 114 immature rats were subjected to hypophysectomy and killed from two to one hundred and two days afterward. Complete hypophysectomy resulted in almost total arrest of increase of body weight and in conspicuous atrophy of the adrenals and testicles and a very slight defect in the weight of the thyroid, these glandular changes being approximately maximal in fourteen, twenty-eight and seven days, respectively. When hypophysectomy was incomplete, regeneration of the anterior lobe in some cases caused, in time, disappearance of

these changes to a variable degree. Loss of body weight sometimes persisted for fourteen to twenty-eight days. Abnormal obesity was associated with accidental injury penetrating the floor of the third ventricle in 5 rats but was absent from 6 rats with apparently similar injuries. One of these obese rats showed, like the others, changes in the adrenals, testicles and thyroid similar to those in completely hypophysectomized animals although two relatively large remnants of the anterior lobe of the pituitary were found microscopically. These remnants were abnormal in showing complete absence of chromophil cells. After complete hypophysectomy microscopic examination showed in the testicles reduction of spermatocytes by fatty degeneration and necrosis, cessation of spermatogenesis, proliferation of basal cells and reduction in the number and size of interstitial cells. In the adrenals the cells of the inner layer of the cortex, particularly of its inner part, were greatly reduced in number by degeneration, partly fatty, and by necrosis; lymphocytic infiltration and pigmentation were slightly increased in the inner part of the inner cortical layer; lipoid was diminished in the inner cortical layer. In the thyroid the signs of active normal secretion and excretion were replaced by those of colloid storage. The degree of these glandular changes at different intervals after operation is described.

FROM AUTHORS' SUMMARY.

CHANGES IN THE ADRENALS IN EXPERIMENTAL HYPERTHYROIDISM. G. HABAN, Beitr. z. path. Anat. u. z. allg. Path. **101**:45, 1938.

Thyroxin was administered to rabbits, cats and guinea pigs over a period of several months. The result was a marked increase in the size and weight of the adrenals. The increase was most pronounced in the cortex, particularly in the zona fasciculata, where an accumulation of lipoids, especially of the anisotropic type, was found. It remains to determine whether the enlargement of the adrenals is the result of hyperthyroidism.

R. J. LEBOWICH.

Pathologic Anatomy

VASCULARIZATION OF ATHEROSCLEROTIC LESIONS. T. LEARY, Am. Heart J. **16**:549, 1938.

It must be concluded that the normal intima is not vascularized, and that blood vessels are found in the intima only in connection with vascular lesions in which repair has taken place. Since the vessels arise as the result of repair processes, i. e., since the lesions are advanced atherosclerotic lesions, the hemorrhages from such vessels must be late phenomena and therefore are not of etiologic importance. Cholesterol found in atherosclerotic lesions is transported to the site in macrophages or as free or ester cholesterol in the blood; it does not arise locally except, perhaps, in small amounts. The laws of nutrition and growth still hold in atherosclerotic lesions.

FROM AUTHOR'S SUMMARY.

SUBACUTE AND CHRONIC GLOMERULONEPHRITIS. E. T. BELL, Am. J. Path. **14**:691, 1938.

Bell studied 181 cases of glomerulonephritis; the condition in 16 was classified as subacute, in 8 as latent chronic, in 117 as chronic azotemic and in 40 as chronic hydropic. In subacute glomerulonephritis the kidneys are not contracted. There is widespread severe glomerular obstruction with well advanced uniform tubular atrophy. There are few hyaline glomeruli. Altogether, 124 cases of chronic azotemic glomerulonephritis were included. Eight cases of latent chronic glomerulonephritis are described, in which death was due to intercurrent disease. Only 1 such case has been reported previously. In such cases there are only a few hyaline glomeruli, and there is little or no tubular atrophy. The glomeruli are all damaged to some degree, their lobules showing hyaline central portions and peripheral capillaries. Thirty cases of chronic azotemic glomerulonephritis are

reported in which there was a history of an initial acute attack. The total duration varied from one and a half years to twenty-six years, with an average duration of ten years. In 15 of the 30 cases the acute attack was followed by a latent chronic stage, varying from one year to twenty-four and a half years in length; in the remaining 15 cases the acute state passed directly into active chronic nephritis. In 30 per cent of the cases the systolic blood pressure was 200 mm. of mercury or higher. There was some degree of chronic passive congestion of the liver in nearly one half of the cases, indicating some degree of heart failure, but there was no evidence that heart failure was ever more than a contributing cause of death, since all the patients had uremia. Heart failure may occasionally have been a contributory cause of edema. Only a single patient had a history of apoplexy. Only a single patient had an attack of coronary sclerosis. Retinitis was found in 35 of 46 patients whose eyegrounds were examined. There is a definite relation between high blood pressure and retinitis. There is no relation between the weight of the kidneys and the duration of the disease or the height of the recorded blood pressure. The kidneys were occasionally of normal size or even enlarged in the terminal stages. Large kidneys contained a high proportion of injured glomeruli with moderately atrophic tubules, while small kidneys consisted largely of hyaline glomeruli with extremely atrophic tubules. Forty cases of hydropic glomerulonephritis (lipoid nephrosis) are reported. In 6 of these the glomerular structure was that of chronic proliferative glomerulonephritis; in 9 others there was a mixture of proliferative glomerulonephritis, and in 9 others there was a mixture of proliferative and membranous glomerular lesions, with the latter in great preponderance. In the remaining 25 cases there were no proliferative lesions. In 6 cases in which young children were concerned there were no visible changes in the glomerular capillaries, and in 3 other cases involving children there were only focal membranous lesions. With 1 exception, diffuse thickening of the basement membranes was present in all persons over 12 years of age. In 4 cases membranous glomerulitis produced such extensive narrowing of the glomerular capillaries that uremia developed. When a patient with pure lipoid nephrosis acquires hypertension and uremia, no new disease is superimposed —there is merely progressive thickening of the basement membranes. Nephrosis is a form of glomerulonephritis in which the glomerular capillaries remain open and allow the blood proteins to escape into the urine. In proliferative glomerulonephritis the capillary lesions are nearly always obstructive in type.

FROM AUTHOR'S SUMMARY.

ACUTE HEMATOGENOUS INTERSTITIAL NEPHRITIS. P. KIMMELSTIEL, Am. J. Path. **14**:737, 1938.

Six cases of acute hematogenous interstitial nephritis are presented, in all of which there was more or less marked isosthenuric oliguria or anuria, with a rise of nonprotein nitrogen in the blood. Twelve other cases of interstitial nephritis, in all of which the condition was found incidentally at autopsy, are included. The morphologic characteristics of this lesion are described. It is emphasized that even in the early stage the interstitial exudate contains plasma cells, lymphocytes, eosinophils and monocytes in addition to polymorphonuclear leukocytes. A distinction between focal and diffuse interstitial nephritis should not be made. The conditions causing interstitial nephritis are listed. In addition to the infectious diseases and septicemia, it is found to follow conditions associated with hemolysis, in particular transfusions of incompatible blood. It is also found in the hepatorenal syndrome associated with infectious and noninfectious injuries of the liver. The correlation of functional disturbances with interstitial nephritis is discussed. It is concluded that the anuria does not result from renal edema by pressure on the tubular apparatus, neither is it caused by blockage of tubules if associated with hemoglobinuria. General or local renal circulatory disturbances are held to be the most likely cause of oliguria and of lack of power of concentration. Hema-

togenous interstitial nephritis is regarded as an allergic hyperergic reaction to foreign proteins or protein split products coincidental rather than causatively connected with hyposthenuric oliguria and anuric uremia.

FROM AUTHOR'S SUMMARY.

THE SILICOTIC NODULE. J. WALSH, Am. Rev. Tuberc. **38**:363, 1938.

Anthracosilicotic dust within the tissues of the lung is always found in cells until after necrosis sets in. These cells are carried in lymph channels to intrapulmonary and hilar nodes. The pulmonary fibroanthracosilicotic nodule begins with thickening of the interstitial tissue around lymphatics, development of fibrous tissue in alveolar walls and destruction of the elastica, which allows the alveoli to collapse. The typical single nodule is smaller than a millet seed and is made up principally of collapsed and coalesced alveolar walls. Later occur hyalinization and necrosis, beginning in the interior. A large nodule is always conglomerate and is made up of the coalescence of innumerable small ones. The silicotic nodules in lymph nodes are also described and differentiated from the pulmonary nodules.

H. J. CORPER.

CHANGES IN THE LIVER IN CHRONIC PASSIVE CONGESTION. E. W. BOLAND and F. A. WILLIUS, Arch. Int. Med. **62**:723, 1938.

The usual histologic picture of the liver in prolonged or recurrent congestive heart failure is that of atrophy or necrosis or both at the centers of lobules. Condensation of reticulum and thickening may or may not be present. The presence of condensation of reticulum alone does not warrant the use of the term "cardiac cirrhosis." True cirrhosis does develop in the course of congestive heart failure, but it does so rarely. No definite criteria were elicited from this study whereby the development or the presence of cardiac cirrhosis can be recognized clinically.

FROM AUTHORS' SUMMARY.

BIOPSY STUDIES OF CEREBRAL CHANGES IN SCHIZOPHRENIA AND MANIC DEPRESSIVE PSYCHOSIS. A. R. ELVIDGE and G. E. REED, Arch. Neurol. & Psychiat. **40**:227, 1938.

Elvidge and Reed investigated, with one of Hortega's silver staining methods, the oligodendroglia in pieces of brain removed during life from patients with schizophrenia, manic depressive psychosis and so-called toxic encephalitis. Two types of swollen oligodendroglia cells were observed. One type contained a nucleus of normal size; in the other type the nucleus was shrunken, pyknotic and vacuolated, and the cytoplasm was increased. The swelling of the oligodendroglia cells remained for a long time, as repetition of biopsies at the end of one or two years showed; i. e., there may be acute and chronic processes present.

G. B. HASSIN.

HERNIATION OF THE BRAIN NOT HERETOFORE DESCRIBED. I. FINKELMAN, Arch. Neurol. & Psychiat. **40**:803, 1938.

Finkelman describes two forms of herniation of the brain not hitherto described in association with tumor or abscess, aneurysm, subdural hematoma and other intracerebral lesions. In one form, which occurred in 80 per cent of the cases of tumor of the frontal lobe, there was bulging of the frontal lobe over the sphenoid ridge and pressure on the tip of the temporal lobe, often on the side opposite the site of the tumor. Occasionally it was bilateral and was associated with hydrocephalus and papilledema. The other form of herniation concerned the gyrus rectus. The posterior part of this gyrus was herniated over the edge of the limbus sphenoidalis, compressed the optic nerve and was practically always associated with choked disk.

G. B. HASSIN.

HUMAN ELLIPTIC RED CORPUSCLES. A. L. FLORMAN and M. M. WINTROBE, Bull. Johns Hopkins Hosp. **63**:209, 1938.

The term "elliptic erythrocyte trait" is defined. Eleven instances of the trait in three families are recorded. A study has been made of the occurrence of these cells in the absence of the hereditary trait. In 89 per cent of a series of 62 nonanemic persons, between 1 and 15 per cent of the cells were elliptic. In a series of 100 consecutive patients with anemia, 98 per cent showed many of these cells, and in 12 per cent of these more than 25 per cent of the cells were found elliptic. Elliptic cells have been found to be more commonly associated with macrocytic than with other types of anemia. In cases of macrocytic anemia these cells seemed to become somewhat more common as the anemia became more severe, but a well defined correlation could not be demonstrated. The anomalous shape seems to be a property of the cells. This shape is characteristic of the mature rather than of the immature red corpuscles of this type.

FROM AUTHORS' SUMMARY.

INFARCTION OF BONES IN CAISSON DISEASE. S. C. KAHLSTROM, C. C. BURTON and D. B. PHEMISTER, Surg., Gynec. & Obst. **68**:129, 1939.

Although destruction of joints and bones has been recognized as one of the manifestations of caisson disease since 1911, the lesions have never been described pathologically. In the 4 cases now reported the condition was of long standing, and there was evidence of multiple infarction of long bones. In 1 case the diagnosis was confirmed by autopsy and in another by biopsy. When the necrotic bone was situated in the epiphyses and bordered on joints, varying amounts of collapse of the weight-bearing portions, invasion and replacement by new bone and calcification of nonsubstituted portions were noted. Articular cartilage overlying affected areas was replaced by fibrocartilage, and more or less extensive arthritis deformans was established. Collapse did not occur when the necrotic bone was situated in the diaphyses or in the epiphyses away from the articular surfaces. On the other hand, there was evidence of some invasion and replacement by new bone and probably complete replacement of some of the smaller foci.

Uncertainty prevails as to whether the necrosis was produced by nitrogen gas obstruction of end arteries or by direct pressure on blood vessels and other tissues after liberation of nitrogen from solution in the fat of the bone marrow or in some other unexplained way. The facts that long bones, which are rich in fatty marrow, were the only ones involved, that fat absorbs relatively large amounts of nitrogen and that nitrogen bubbles would be absorbed slowly from the marrow favor the view of direct pressure of the gas on vessels and other tissues within the bone. On the other hand, the extensive involvement of the diaphyses in some cases without lesions in the epiphyses, especially of the head of the femur, without involvement of the diaphyses or without continuity of affected regions in epiphysis and diaphysis are points in favor of embolism by nitrogen gas or some other form of intravascular obstruction. Experimental air embolism of the lower limbs of dogs failed to produce bone necrosis.

FROM AUTHORS' SUMMARY (WARREN C. HUNTER).

EPITUBERCULOSIS. R. H. FISH and W. PAGEL, J. Path. & Bact. **47**:593, 1938.

A survey of the literature on "epituberculosis" is made, and the observations at necropsy in 3 new cases are recorded. The process appears to be related to a primary caseous pulmonary focus but may also be observed in postprimary tuberculosis of adults. It is concluded that in cases in which a condition has been clinically diagnosed as "epituberculosis" a noncaseating tuberculous tissue reaction may be found and may be regarded as the normal response of tuberculoallergic lung tissue to contact with material containing tuberculous protein but few live

tubercle bacilli. This type of lesion should be distinguished from collapse due to external bronchial compression, which may give rise to a comparable clinical picture. However, mixed cases are common. FROM AUTHORS' SUMMARY.

THE THYROID GLAND OF THE CRETIN AT BIRTH. J. EUGSTER, Beitr. z. path. Anat. u. z. allg. Path. **100**:392, 1938.

Prompted by the almost complete absence of knowledge of the anatomic state of the thyroid gland of the cretin at birth, Eugster investigated 4 such glands. The findings were not uniform, 2 showing a pronounced diffuse parenchymatous struma, and a third distinct atrophy and sclerosis. In the fourth there was a slight, probably goitrous enlargement. The difficulty of establishing a clinical diagnosis of cretinism at birth is discussed in the light of the pathologic changes.

R. J. LEBOWICH.

Microbiology and Parasitology

THE PRESENT INCIDENCE OF TRICHINELLA SPIRALIS IN MAN AS DETERMINED BY A STUDY OF ONE THOUSAND AND SIXTY UNSELECTED AUTOPSIES IN ST. LOUIS HOSPITALS. T. B. POTE, Am. J. M. Sc. **197**:47, 1939.

Muscle tissues obtained at 1,060 autopsies were trichinous to the extent of 15.4 per cent. There had been no symptoms suggesting trichinella infection. In 95 per cent of the specimens the trichinellas were dead, calcified and more or less disintegrated; in 5 per cent they were demonstrated to be alive by feeding the infected material to rats and recovering live larvae. Very heavy infections were to be observed in cases in which the host gave a history of having always enjoyed good health. In a patient dying at the age of 84 as the result of an accident autopsy did not show that the infection had contributed to the cause of death.

In a series of 1,500 hog carcasses examined for trichinella, 0.8 per cent were found to be infected, an observation which gives rise to the question whether man is getting all of his trichinella infection from pork. It may be concluded that man's infection comes from pork derived from the small packing plants, which are not supervised by meat inspection services. It is to be noted that the infections that sporadically occur are from pork from establishments not under federal inspection.

It can be definitely concluded that Trichinella spiralis infection of man is not a serious lethal factor in Missouri, since in 163 instances of infection it was not held to have been the cause of death or to have contributed to the cause of death.

FROM AUTHOR'S SUMMARY.

INTOXICATION AND RESISTANCE TO INFECTION. K. L. PICKRELL, Bull. Johns Hopkins Hosp. **63**:238, 1938.

Alcoholic intoxication maintained at the point of stupor destroys resistance to pneumococcic infection in the rabbit. Even animals rendered highly immune by intravenous injections of antipneumococcus serum were deprived of their immunity. The loss of resistance to the infection appears to be due to the fact that intoxication profoundly inhibits the vascular inflammatory response as long as it is maintained, and in the absence of capillary dilatation and emigration of the leukocytes, leukocytic margination at the site of infection is negligible, and the bacteria therefore proliferate uninterruptedly. Similar experiments show that ether or avertin anesthesia has as marked an inhibitory effect on the inflammatory response and produces as marked a loss of resistance to infection.

FROM AUTHOR'S SUMMARY.

DETECTION OF THE VIRUS OF POLIOMYELITIS. S. D. KRAMER, B. HOSKWITH and L. H. GROSSMAN, J. Exper. Med. **69**:49, 1939.

Five strains of the virus of poliomyelitis were recovered from nasal washings and feces. Four strains were of human origin; the fifth strain came from a monkey put to death at the height of the disease. Of the human strains, the first was isolated from the feces of a 14 year old child seven days after the onset of illness. The second strain was from the nasal washings of a 6½ year old child five days after the onset of illness. One of these strains was obtained from nasopharyngeal washings and the other from feces. The single monkey strain was isolated from the upper intestinal segment, and this appears to be the only instance of isolation of a strain of this virus from such a source recorded in the literature. The authors state that the detection of the virus in the nasal washings of 2 additional patients who were convalescent lends further support to the belief that the virus of poliomyelitis is spread by human contact. Furthermore, the recovery of the virus from the gastrointestinal tract with as great or greater frequency than from the upper respiratory tract need not, it appears to these authors, alter the present concept of the mode of entrance of the virus into the body, namely, by way of the upper respiratory tract. If the presence of the virus in the upper respiratory tract is conceded, the passage of nasal and oral secretions into the gastrointestinal tract by reflex swallowing would serve to explain the presence of the virus in this tract. It might be further predicated that since the gastrointestinal tract functions as a temporary reservoir for secretions from the upper respiratory tract, the intestine should, after a time, contain the virus in higher concentration than any single sample of secretion obtained from the upper respiratory tract by nasal washing. Failures to detect the virus in the gastrointestinal tract are perhaps more indicative of the inadequacy of the procedures for the detection of the virus than of the absence of the virus. The recovery of the virus from the feces seven and nine days after the onset of illness takes on added significance. It indicates first that the virus withstands the gastric acidity which under normal physiologic conditions tends to keep gastric contents relatively free from bacteria. It further suggests that improper disposal of feces from patients with poliomyelitis may have serious consequences for public health, particularly in smaller communities, where the inadequate disposal of sewage may result in contamination of surrounding beaches or even local water systems.

FROM AUTHORS' SUMMARY.

STUDIES IN THE METABOLISM OF COCCIDIOIDES IMMITIS (STILES). R. A. STEWART and K. F. MEYER, J. Infect. Dis. **63**:196, 1938.

The resistance of Coccidioides to desiccation may be an important factor in the dissemination of the organism in the dry, warm climate of the San Joaquin Valley, in California. Under conditions of drying the chlamydospore is formed, and it is apparently not possible to infect animals in the absence of this spore.

A synthetic medium has been devised which possesses selective properties of value in the isolation of Coccidioides from the soil and a wide variety of clinical materials.

The metabolism of Coccidioides immitis and Blastomyces dermatitidis did not present any significant difference in either a protein-rich medium or a synthetic medium.

Contrary to the literature, dextrose is assimilated by both Coccidioides and Blastomyces. This throws doubt on the accuracy of the statements relative to consumption of sugar based on changes in the p_H of a medium. Changes in the p_H occur independently of the consumption of dextrose and are to be explained by variations in the consumption of other constituents in the medium.

The metabolism of C. immitis and B. dermatitidis differs from the metabolism of bacteria and certain protozoa in that the consumption of dextrose does not suppress the production of ammonia. The assimilation of dextrose favors the production and the assimilation of ammonia.

Fungi are biologic entities or individualists and are not always amenable to the principles established by bacteriologic procedures—they require their own type of medium. The medium should be chemically defined whenever possible, so that morphologic and physiologic characteristics may be referred to an environment that can be duplicated anywhere. This type of medium offers the best "common denominator" for the correlation of results reported by widely scattered investigators.

FROM AUTHORS' SUMMARY.

EPIDEMIC INFLUENZA VIRUS. T. P. MAGILL and F. T. JUN, Brit. J. Exper. Path. **19**:273 and 284, 1938.

Cross neutralization tests performed with 24 strains of influenza virus are reported, and the significance of the findings is discussed. Although marked differences between some strains and striking similarities between others permits rough grouping, the characteristics of one group tend to merge into those of another. The strains which most closely resemble one another are in general those from the same epidemic of influenza, but serologically different strains may be recovered from the same epidemic. The specific effect of a given serum can be demonstrated by its action in tissue cultures as well as by the usual mouse protection test. So far as it has been possible to determine, the antigenic differences between strains have not resulted from variations occurring in the course of animal passage or artificial cultivation. It is suggested that the structure of the virus of epidemic influenza is a mosaic of antigens, numerous strains containing the same antigens arranged in such a way that each strain or group of strains is characterized by its own peculiar antigenic pattern. It is further suggested that all strains may not contain all the antigenic components of all other strains.

Cross immunity tests were carried out with 16 strains of epidemic influenza virus and 1 strain of swine influenza virus in actively immunized mice. It was found that antigenic differences in strains such as those demonstrated by serologic tests in the preceding paper are reflected in a lack of cross immunity. In general, strains obtained in the same year tend to be closely related immunologically, although individual differences exist. While quantitative factors are undoubtedly involved in the problem of cross immunity between strains, qualitative differences can be shown in strains of equal virulence. The differences scarcely seem to warrant a designation of types, since most strains of human origin are closely related. The importance of the serologic and immunologic differences between strains from the point of view of the problems of epidemiology and prophylaxis is discussed.

FROM AUTHORS' SUMMARY.

THE STRUCTURE OF "ROUGH" AND "SMOOTH" COLONIES. K. A. BISSET, J. Path. & Bact. **47**:223, 1938.

The structure of a morphologically rough colony of a bacillus is essentially similar to that of the medusa head colony of the anthrax bacillus. The bacilli lie close together in threads. The bacilli composing a smooth colony are all separate and show no characteristic arrangement. Between these extremes are a number of intermediate forms. These variations are paralleled by similar forms found among the streptococci. The usually described SR variations appears to consist of two separate changes, one affecting the morphologic appearance of the individual organism, the other connected solely with the nature of its surface material. These may occur separately, though a considerable degree of relationship appears to exist. The structure of a colony depends ultimately on physical factors and varies mainly with the degree of attachment shown by the component organisms.

FROM AUTHOR'S SUMMARY.

SURVIVAL OF RICKETTSIA PROWAZEKI IN LICE. J. STARZYK, Arch. Inst. Pasteur de Tunis **27**:263, 1938.

Typhus rickettsias were found to survive as long as six days in human serum diluted 1:1 and maintained at 5 to 7 C., and a lesser period under other conditions, as tested by the percentage of lice infected. Also when kept cold and dried under a vacuum they survived six days. The results not only indicate a useful point in preserving rickettsias for use in studies or in the production of vaccines or in shipping but may have epidemiologic significance. The survival of the infective agent in clothing and on premises for some days after the death of infected lice raises new questions with regard to dissemination and control of typhus.

M. S. MARSHAL.

Tumors

CANCER IN RATS TREATED WITH OESTRONE. C. S. MCEUEN, Am. J. Cancer **34**: 184, 1938.

Rats were treated for long periods with an estrogen (theelin, or estrone), usually in conjunction with various forms of local irritation. The occurrence of tumors histologically diagnosed as malignant was found to be more frequent in these animals than in controls or in rats of the same colony subjected to other procedures, used for breeding or set aside without treatment.

FROM AUTHOR'S SUMMARY.

ACTION OF COLCHICINE ON A TRANSPLANTED MALIGNANT NEOPLASM. F. J. LITS, A. KIRSCHBAUM and L. C. STRONG, Am. J. Cancer **34**:196, 1938.

A malignant transplanted lymphoid tumor was subjected to a subcutaneous injection of 0.025 mg. of colchicine in 0.1 cc. of distilled water every three days, each injection being made at some distance from the tumor. By this treatment the tumor was caused to regress, and the animals survived fifty and one-half days after the implantation of the tumor, as compared with thirty-one and one-half days for untreated controls (13 controls and 14 test animals). Histologic studies showed that the regression was due to repeated so-called "caryoclastic shocks"; the lymphocytes of the tumor became pyknotic and died. The reticular cell elements of the tumor were most resistant to treatment by colchicine. There were also some foci of resistant lymphocytes. Tumors which recurred seemed to arise by proliferation of the reticular cells of the regressed growth. Both the lymphoid and the reticular cells of the lymphosarcomatous growth studied may be considered malignant. A difference in susceptibility to colchicine exists between normal and malignant lymphocytes, the malignant cells being much more susceptible. Normal lymphocytes of the thymus are destroyed quite readily by the drug, in contrast to the more resistant lymphocytes of the spleen and lymph nodes.

FROM AUTHORS' SUMMARY.

STRUCTURAL DEVELOPMENT OF GLIOMA. H. J. SCHERER, Am. J. Cancer **34**:333, 1938.

A systematic study of the structural evolution in 100 gliomas has been made by means of large pyroxylin (celloidin) sections taken in multiple planes and including the whole tumor with its surrounding tissue. Glioma structures depend on the preexisting nerve structures (secondary structures) or on primary architectural properties inherent in the glioma cells (proper structures). The structure of a glioma may be influenced, also, by connective tissue which invades the tumor (tertiary structures). When the cells of the glioma are uniformly and evenly distributed, the structure is described as amorphous. The following secondary structures are described: perineural or neuronophagic growth, perivascular growth, superficial growth, perifascicular, intrafascicular and interfibrillary growth, and

elective growth in gray and white matter. The proper structures are classified as canalicular, papillary, fascicular and symplastic structures and ribbon and palisade formations. The secondary structures play a much more important role in the architecture of most gliomas than do the proper structures. In certain rare gliomas, having a strictly expansive type of growth (ependymomas), secondary structures do not develop. Secondary structures do not always develop in the infiltrating gliomas, sometimes because of the extremely rapid destruction of the preexisting tissue (medulloblastomas, certain glioblastomas), but certain other infiltrating tumors remain amorphous in spite of the perfect conservation of the preexisting tissue (typical astrocytomas). Three stages of structural evolution are encountered in the majority of gliomas. Various secondary structures are formed first, followed by an amorphous arrangement and finally by the various proper structures. There are marked differences in the structural development of gliomas. These variations must be considered as an expression of the fundamental biologic differences of the tumors themselves and must be taken into consideration in any future classification together with the localization, mode of extension and cellular appearance. Gliomatous structures differ in the same tumor depending on the area invaded (different secondary structures) and on the age of the tumor (different stages of the structural evolution). Stable structures in gliomas are rather rare. At present differences between gliomas in respect to structural evolution are unexplained. The etiologic explanation of the curious secondary structures is also unknown. A review of the literature suggests that carcinomas also show differences of structural development in the sense that either proper structures or secondary structures may appear to predominate. This suggests that there are broad and general laws which determine the morphologic evolution of all neoplasms.

FROM AUTHOR'S SUMMARY.

ANTERIOR PITUITARIES OF OLD RATS. J. M. WOLFE, W. R. BRYAN and A. W. WRIGHT, Am. J. Cancer **34**:352, 1938.

The anterior lobes of the pituitaries of old male and female rats over 18 months of age were studied and compared with those of younger animals. In those from the old rats it was found that the levels of eosinophils were significantly lower and those of the chromophobes were significantly higher than the levels of the corresponding cells in the younger animals. No important difference was found in the levels of the granular basophils in young and old rats, but nongranular basophils were significantly more abundant in the younger ones. In one strain of rats the incidence of benign mammary fibroadenoma in the old females was quite high, while in the other two strains it was much lower. In all three strains it was found that there was a tendency for the levels of the eosinophils to be lower in those rats bearing mammary tumors than in rats without tumors. There was no difference in the levels of the granular basophils in the two groups, but the nongranular basophils were more abundant in rats without mammary tumors. Chromophobes were generally more abundant in rats with mammary tumors. It was found that adenomatous changes of the anterior lobe of the pituitary occur frequently in old rats, particularly in females. Adenoma associated with marked hemorrhage and classified as hemorrhagic adenoma, chromophobe adenoma and an adenomatous nodule that was believed to be a forerunner of true adenoma are described. All quantitative data were handled by statistical methods.

FROM AUTHORS' SUMMARY.

MODE OF ACTION OF METHYLCHOLANTHRENE. W. R. EARLE and C. VOEGTLIN, Am. J. Cancer **34**:373, 1938.

The action of methylcholanthrene has been studied on rat and mouse fibroblasts. Under the usual cultural conditions methylcholanthrene severely retarded the growth and caused degeneration of the cultures, the effect being roughly proportional to the concentration of the reagent and the time of exposure. This action

was most accentuated with concentrations of 2 mg. of crystalline methylcholanthrene per cubic centimeter but was also demonstrated with concentrations as low as 0.0002 mg. per cubic centimeter. In a single extensive series of mouse fibroblasts studied in detail, in which wavelengths of light covering the absorption spectrum of methylcholanthrene down to 490 microns were eliminated, the same retardation of growth was observed as in the other series, but there was less cell degeneration. The retardation of growth cannot be attributed to the action of an impurity in methylcholanthrene. One series of cultures was studied with 1,2,5,6-dibenzanthracene. While conditions were not so carefully controlled as with the other series, the cultures showed a marked injurious action of this substance. In no instance in the experiments reported was any change observed in the cultures which could be considered as causing the normal fibroblasts to assume a morphologic character similar to that of the cells which were studied in growths arising as a consequence of the subcutaneous and intramuscular injection of methylcholanthrene into rats. In no instance was there noted any stimulative action of either the dibenzanthracene or the methylcholanthrene. The significance of these results is discussed in relation to those published by others.

FROM AUTHORS' SUMMARY.

VITAMIN A AND TUMOR MITOCHONDRIA. A. GOERNER and M. M. GOERNER, J. Biol. Chem. **123**:57, 1938.

Vitamin A was not present in the mitochondria of tumor cells in either the Flexner-Jobling rat carcinoma or the R-39 sarcoma despite the fact that the hepatic mitochondria of both types of tumor-bearing animals contained a normal store of the vitamin as well as of total lipid. The total lipid content of the mitochondria of the tumor cell was fairly large in both experimental tumors but was higher in the Flexner-Jobling rat carcinoma than in the sarcoma. The results indicate that an increase in the vitamin A intake can have little effect on the growth processes of tumor cells.

R. J. LEBOWICH.

NEOPLASM OF MONOCYTES. J. FURTH, J. Exper. Med. **69**:13, 1939.

A transmissible neoplasm of mice characterized by malignant cells resembling histiocytes (monocytes) is described. The morphologic aspects of these cells and the microscopic appearance of the neoplasm are similar to those of the human neoplasm formed by histiocytes. The malignant histiocytes form tumor-like masses in the liver and spleen and infiltrate these and other tissues. When the disease is far advanced, they are present in small numbers in the blood of many mice. The malignant cells have scant phagocytic ability. The fixed cells of the host (endothelial cells and fibroblasts) have no significant part in the production of the lesions of the disease. Transmission is readily accomplished when material containing the malignant histiocytes is used for inoculation but fails in their absence. Attempts to demonstrate a cell-free transmitting agent have been unsuccessful.

FROM AUTHOR'S SUMMARY.

SOLUBLE ANTIGEN OF MYXOMA. T. M. RIVERS, S. M. WARD and J. E. SMADEL, J. Exper. Med. **69**:31, 1939.

The soluble antigen of myxoma is a heat-labile protein which has an isoelectric point near p_H 4.5 and is precipitated from half-saturated solutions of ammonium sulfate. It can be partially purified by methods of differential precipitation based on variations in the p_H and electrolyte concentration. In rabbits receiving the labile soluble substance of myxoma homologous precipitins develop, and their serum agglutinates elementary bodies of myxoma, provided the dermal pulp from which the bodies are obtained contains the soluble substance; neutralizing antibodies do not appear, however, and the animals are not resistant to infection with the virus of myxoma. Elementary bodies of myxoma appear to

have a heat-stable agglutinogen which operates when brought in contract with serum from animals recovered from myxoma but which shows little action, if any, when in contact with anti-soluble-substance serum.

FROM AUTHORS' SUMMARY.

CARCINOGENIC EFFECT OF PAPILLOMA VIRUS. J. G. KIDD and P. ROUS, J. Exper. Med. **68**:529, 1938.

A considerable variety of tumors, both benign and malignant, result from the localization of the virus of rabbit papilloma in skin which has been prepared by repeated tarrings. They appear only in animals highly susceptible to the action of the virus, and are more likely to be engendered by highly pathogenic inoculums. No evidence has been found that differences in the potentialities of the virus entities are responsible for the diversity of the growths. This is referable to changes in the epidermal cells; and much more preliminary tarring is required to produce these changes than suffices to cause localization of the virus out of the blood stream, with a resulting papillomatosis of the ordinary sort. The character of the individual anomalous tumors depends in some degree on the extent of the preparatory changes in the cells, malignant growths being more frequent when the epidermis has been tarred for a relatively long period. All are focal or punctate in origin, and they exhibit their peculiar characters from the first, these characters not being due in any case to secondary alterations in ordinary papillomas. Tarring after the virus has localized in the epidermis does not significantly increase the number of growths. Their development is the outcome of the state of the cells at the time of virus infection. Tarring exerts important influences in addition to changing the cells in such a way that unusual tumors result from the action of the virus. The procedure is notably effective in determining localization of the virus out of the blood stream; it enables the virus to produce growths when otherwise it would not do so though present in the tarred skin; it stimulates the proliferation by which the tumors are engendered; it makes them disorderly and aggressive, and it hastens the anaplasis of such of them as are malignant. It has similar effects on the tar tumors, as will be demonstrated in a subsequent paper.

FROM AUTHORS' SUMMARY.

FIBROMA VIRUS INFECTION IN TARRED RABBITS. C. G. AHLSTRÖM and C. H. ANDREWES, J. Path. & Bact. **47**:65, 1938.

Rabbits which have been given intramuscular injections of tar respond in an abnormal way to the virus of infectious fibroma (OA strain). In tarred animals the regression of lesions produced by intradermal or subcutaneous inoculations of the virus is much delayed. In some tarred rabbits the subcutaneous tumors may even grow progressively and invasively until the animals die, coming to resemble true neoplasms both clinically and histologically. After intravenous inoculation of the virus into tarred rabbits, generalized fibromatosis commonly develops and may be fatal. In tarred rabbits antibodies to the virus develop, and the animals become immune to reinoculation in the same way as controls. A single dose of tar given on the same day as the virus produces the effects described; a series of inoculations is unnecessary. Benzpyrene and other carcinogenic hydrocarbons produce effects similar to those of tar, but it is not certain how far these effects are specific for carcinogenic substances. No effect of tar was detected in infections with inflammatory (IA) variant strain of the virus of fibroma nor with the virus of vaccinia.

FROM AUTHORS' SUMMARY.

A SUPRASELLAR TUMOR IN A DOG. E. G. WHITE. J. Path. & Bact. **47**:323, 1938.

A suprasellar tumor in a dog, considered to be a craniopharyngioma of unusual structure, is described. The animal showed during life a syndrome resembling dystrophia adiposogenitalis in man.

FROM AUTHOR'S SUMMARY.

MORPHOLOGY OF EPULIS. L. GERY and O. NOEPPEL, Bull. Assoc. franç. p. l'étude du cancer **27**:137, 1938.

Gery and Noeppel define epulis as a sessile or a pedunculated outgrowth of the gum, originating from its soft tissue. They studied 240 such growths seen between 1919 and 1937 at the Institute of Pathology in Strasbourg, France, and found 230 of them to be hyperplastic tumors of the mesenchyma or, rarely, of the alveolodental ligament. All were benign, though some recurred locally. Histologically they could be divided into four not sharply separated groups: the inflammatory, the vascular or angiomatous, the fibrous and the giant cell group. Transitions among them were so frequent that a histologically homogeneous form was rare. The inflammatory epulis consisted of granulation tissue rich in blood vessels and in an exudate that sometimes was leukocytic but often plasmocellular. The vascular epulis showed excessive formation of blood vessels, which lacked the regularity and arrangement that are characteristic for granulation tissue. The fibrous epulis was made up predominantly of fibroblasts with varying amounts of intercellular fibers. Two types of cells determined the giant cell epulis: a small mononuclear cell resembling a fibroblast, but easily differentiated from it, and the well known multinucleated giant cell, similar to the bone marrow giant cell. The small cells, which could be shown to be connected with each other by means of protoplasmic outgrowths, are considered by Gery and Noeppel to be the fundamental unit of the growth. The hypotheses concerning the formation of the giant cells are discussed in detail. Their mesenchymal origin and lack of any relation to the bone marrow giant cells are emphasized, and the name "histioplaxe" is suggested for them. In all the forms of epulis deposits of collagenous masses were found, as well as ossification. The latter is explained as the function of the histiocyte in the sense of Maximow's polyblast. The deposition of calcium in the preformed osteoid tissue, as well as calcification without bone formation, is brought into relation with the observation that not infrequently decalcification of the alveolar process was recorded with transport of calcium, much as in callus formation. New-formed cartilage was observed very rarely. The authors emphasize the evidence that speaks against the origin of the epulis from bone In their opinion, the epulis is formed by the reticulum in response to an unknown irritating. factor The histiocyte with its formative potentialities is fully capable of explaining the morphologic variations of the epulis and the frequent observation of phagocytosis. I. DAVIDSOHN.

Medicolegal Pathology

EPENDYMAL CYSTS CAUSING SUDDEN DEATH. K. BÖHMER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **30**:59, 1938.

Two deaths, supposedly resulting from accidents, were explained at autopsy by tumor occlusion of the aqueduct of Sylvius. In each instance the tumor was a cyst of ependymal origin, covered with connective tissue and lined by a layer of epithelium. The cyst content was rich in cholesterol and lipochrome. The first patient was a woman 46 years old who had complained of headaches for two months. She fell off a merry-go-round, struck her head on an iron rail and became unconscious for twelve hours. In the interval of two weeks between the time of the accident and her death she had headaches almost constantly. She died suddenly. The tumor was found in the third ventricle and was the size of a hazelnut. The accident two weeks before death was regarded as a result of temporary occlusion of the aqueduct by the tumor.

The second death occurred in a cyclist a day after he had been found unconscious beside a cycle path. After regaining consciousness, he thought he had been struck on the shoulder by another rider and that he had lost his balance and fallen. However, he had sustained no serious injury, and his bicycle was undamaged and had been set against the curb by him. The tumor in the aqueduct was only 2 mm. in diameter. The lining of the third ventricle was roughened.

A reconstruction of the course of events seemed to justify the assumption that the man had exerted himself while riding uphill, disturbed the cerebrospinal fluid balance in the brain and caused the tumor, which it is assumed had theretofore floated freely, to clog the aqueduct. With the first evidences of cerebral compression from the lateral ventricles, the man dismounted, leaned his bicycle against the curbing and then sank to the ground in a stupor.

Böhmer cites cases reported by Beutler, Sjövall and Weissenburg to substantiate his interpretation of the cases reported. The theories of the cause of death in occlusion of the third ventricle are discussed briefly. GEORGE RUKSTINAT.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, April 10, 1939

EDWIN F. HIRSCH, *Secretary*

RELATION OF CIRCULATING PRECIPITINS TO THE ARTHUS PHENOMENON. PAUL R. CANNON and CHARLES E. MARSHALL.

The uncertainty as to the role of circulating precipitins in the Arthus phenomenon has been due mainly to the use of inadequate methods for determining the precipitin content of serum. Dilutions of the antigen have been used in most studies, although this procedure is a titration of antigen rather than of antibody. We have studied the problem as follows: Collodion particles to which crystalline egg albumin had been adsorbed were added to serial dilutions of serum from rabbits immunized against crystalline egg albumin. By centrifugation and resuspension the antibody strength of such serums can be determined readily and correlated with the varying degrees of cutaneous reactivity to intradermal injections of crystalline egg albumin during immunization, after desensitization, after passive transfer and in other conditions. The results indicate that the Arthus phenomenon is associated invariably with the presence of specific precipitins in the serum of the animal and that the intensity of the inflammation parallels the precipitin content of the serum.

DISCUSSION

EDWIN F. HIRSCH: Do you have an explanation for the apparent lag in the appearance of the cutaneous sensitivity in reference to the appearance of the precipitin content of the blood?

I. DAVIDSOHN: This is a significant contribution since Opie's report.

PAUL R. CANNON: The precipitin content of the blood must be at a certain level—a concentration effective in dilutions of 1:240 or 1:480—before the skin reaction will occur. Then the skin reaction increases in proportion.

OBLITERATION OF THE PLEURAL SPACE FOLLOWING PNEUMONECTOMY. F. J. PHILLIPS and W. E. ADAMS.

Cancer of the lung is being diagnosed earlier in the course of the disease because of increased diagnostic acumen and improved laboratory methods. The technical procedure of pneumonectomy is better as a result of the work of Graham, Churchill, Rienhoff and others. The anatomic and physiologic changes following pneumonectomy are not well understood. Following pneumonectomy the human pleural space becomes filled with a fibrous tissue shell by organization of the accumulated serofibrinous exudate. Mediastinal shift does not appear to be a dangerous hazard in these patients. The details of successful total pneumonectomy in a man aged 58 with squamous cell carcinoma of the left bronchus are presented. Nine months later he died from generalized peritonitis following rupture of the appendix. The fibrous tissue shell in the left side of the chest was 1 to 3 cm. thick and contained a brown turbid fluid. Protocols of experiments in dogs on subtotal collapse and pneumonectomy demonstrate obliteration of the pleural space by hyperdistention of the remaining inflated lung tissue.

REGIONAL FIBROCYSTIC DISEASE. CARROLL O. ADAMS.

Regional fibrocystic disease is a rare condition which is usually confused with other extensive cyst formations in bones. This report is the conclusion of a study

of 10 cases available for study at the University of Chicago Clinics. Two patients had involvement of an upper extremity only. These had a monomeric distribution, with involvement of the humerus and radius and, in 1 case, of the first metacarpal bone, without involvement of the ulna or the other bones of the hand. One patient had a small lesion in the humerus and massive lesions of the pelvis, femur, tibia and fibula on the same side. In another patient the ilium and femur on each side and the tibia on the left side were the loci. The other 6 patients had involvement in a lower extremity only.

Regional fibrocystic disease is probably congenital. This explains the varied extensiveness, the common segmental distribution and the usual onset of symptoms in childhood. The symptoms are mild aching pain following trauma, but pathologic fracture may be the first manifestation. These fractures usually heal, but delayed repair and malunion are common. Refracture is not rare, and nonunion occurred in 1 patient. The levels of the blood calcium and phosphorus are normal.

The roentgenograms usually showed the diaphysis expanded, especially in regions of previous fracture. The cortex was usually thin, and over the convex side of bowed long bones it was sometimes barely perceptible. In such regions fractures, complete and incomplete, may occur repeatedly. Occasionally, regenerative tissues developed in these weakened places. In the medullary region the normal trabecular pattern was replaced by irregular heavy trabeculae between which there might be decreased density and complete absence of trabeculae. In 2 patients with involvement of an upper extremity the humerus had this typical appearance, while the cortex of the radius was thin, and the entire medullary region was reduced in density and was without trabeculation. The epiphyses usually appeared normal.

The cortex of the bone was thin, and some of it could be sectioned with a knife. The marrow was replaced by yellow, gray or white fibrous tissue. Unlike the localized type, there were few cystic cavities, and the regions which appeared cystic in the roentgenograms were filled with fibrous tissue. In the regions which appeared devoid of trabeculae in the roentgenograms there were a few fine irregular trabeculae scattered throughout the fibrous tissue. Microscopically, the fibrous tissue was usually mature and dense. The cystic cavities present contained gelatinous material and were lined by compact fibrous tissue. The trabeculae had many irregular cement lines and occasionally some osteoid tissue.

Treatment was limited to the preservation of function, the prevention or correction of deformity and the curettement of regions with severe persistent pain.

BRONCHIAL OBSTRUCTION PRODUCED BY CARDIOVASCULAR ANOMALIES. PAUL HOLINGER and A. H. ANDREWS.

Tracheal or bronchial obstructions produced by cardiovascular anomalies are relatively rare. They have striking and frequent unusual clinical manifestations which generally are explained only by the postmortem examination. As in any bronchial obstruction, whether caused by an intrabronchial, an endobronchial or an extrabronchial lesion, that caused by a cardiovascular anomaly manifests itself by obstructive emphysema or atelectasis. Four representative examples were presented.

The first was in a 4½ month infant who had died of asphyxia from compression of the trachea. Postmortem examination revealed the aorta divided into two arches which surrounded and constricted the trachea where the branches passed backward and united posteriorly. The second patient, an infant of 5 months, had a partial bronchial obstruction with marked obstructive emphysema caused by a transversely placed distended right auricle which compressed the bronchus against the thoracic aorta. Numerous other cardiac anomalies, including atresia of the tricuspid valve, were present. In the third patient an atelectatic left lower pulmonary lobe was due to compression of the lung by a congenitally enlarged heart. In the fourth patient marked obstructive emphysema of the right lung had resulted because the aorta, by passing to the right of the trachea instead of to the left, had partially compressed the right main bronchus.

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, May 8, 1939

EDWIN F. HIRSCH, *Secretary*

STUDIES ON THE SEX RATIO: (A) THE METABOLIC GRADIENT AS A CONDITIONING FACTOR; (B) THE SEX RATIO AND RESISTANCE. WILLIAM F. PETERSEN and MILDRED NORVAL.

Apart from obvious differences, one basic biologic difference between the sexes resides in the fact that the male organism throughout its entire life span is geared to a higher oxidative rate; i. e., it needs more energy in order to exist. The biologic inference seems obvious, for such a situation proves beneficial in providing greater opportunity for female survival and therefore for racial survival. If a large share of the energy expenditure is devoted to the maintenance of body temperature, the male may be expected to be less resistant to cold, first because of less fat tissue insulation, second, because of lesser opportunity to accumulate reserves than is afforded the female under similar conditions, and, third, because of greater demand on reserves during unusual stress or strain, such as cold, fatigue and illness.

An examination of the day by day sex ratio of persons dying in Detroit in the years 1934, 1935 and 1936 indicates that the ratio of males to females increases when masses of cold air pass over the region. On the other hand, during periods of mortality from undue heat (Detroit in June 1936 Chicago in July 1934) the trend of this ratio is relatively lower.

Despite the advantage of a generally lower basal metabolic rate for the female, it seems that adjustment to undue heat in general is more difficult for the female because of her greater peripheral insulation. This apparently finds expression, too, in the general experience of white persons domiciled in the tropics, where the female suffers more acutely and ages more rapidly than the male.

DISSEMINATED GIANT CELL REACTION; POSSIBLY PRODROMAL MEASLES. WALTER A. STRYKER.

In a 27 month old child dying of pneumonia multinucleated giant cells were found in the pulmonary alveoli, the bronchi, the sinuses and medullary cords of peribronchial lymph nodes, the lumens and walls of bronchial mucous glands, the interstitial connective tissue about lymph nodes and mucous glands, the spleen and the lymphatic tissue of the ileum. These cells were morphologically like those described as specific for the prodromal stage of measles. A history of possible contact with measles was obtained. Attempts to demonstrate inclusion bodies were inconclusive.

DISCUSSION

I. DAVIDSOHN: The report is interesting, especially because of the widespread occurrence of the giant cells. The variety of giant cells is most amazing. May the nature of the disease be diagnosed on the basis of the microscopic structure of the tissues? I am inclined to think that the tissue structure probably indicates measles.

WALTER A. STRYKER: This case is not presented as a proved instance of giant cells in the prodromal stage of measles. The best evidence would be the demonstration of inclusion bodies in the giant cells in the prodromal stage as in the active stage of measles.

DYSGERMINOMA OF THE TESTICLE. J. D. KIRSHBAUM and MAURICE B. JACOBS.

Thirty malignant testicular tumors were selected for study in the department of pathology of the Cook County Hospital; 26 of the tumors were classified as dysgerminoma and 4 as mixed—i. e., they contained dysgerminoma tissues

associated with cell cords and acini, the cell type, however, being uniform. The latter 4 tumors may be considered as representing an intermediate stage of a more highly specialized type of the dysgerminoma. Robert Meyer introduced the term "dysgerminoma," in 1930.

All of the tumors had a single type of cell, which, according to Robert Meyer, originates from the undifferentiated germ cell of the gonads. This conception explains the occurrence of similar tumors in ovaries. In size the tumors ranged from that of a walnut to that of a grapefruit. The tissues were moderately firm, encapsulated, homogeneous and gray-white. There were hemorrhages in 20 and necrosis in 25, of which 5 had caseation necrosis resembling tuberculosis, with tubercles but no Langhans giant cells. The tumor cells were large and polygonal, contained ample cytoplasm and had round or oval, deeply stained nuclei. Nucleoli were frequent, and cells with atypical mitosis were abundant. The arrangement of the cells was diffuse in 20 tumors, in the form of alveoli in 6 and after a papillary pattern in 4. Some had large irregular nuclei; often there occurred one that was multinucleated. The stroma frequently contained small lymphocytes, and narrow connective tissue trabeculae extended between alveolar masses of cells.

The youngest patient was 18 years of age; the oldest was 63. Fifty-seven per cent of the patients were in the third and fourth decades of life, while 58 per cent of the 12 patients examined post mortem were in the third decade. Twenty-seven patients were white and 3 were colored. The left and the right testicles were affected in equal numbers. Eleven of the patients gave a history of trauma preceding the onset of the tumor. Only 9 patients complained of pain on admission, while swelling was mentioned by 23. In 10 patients there was a loss of weight ranging between 20 and 35 pounds (9 and 16 Kg.). Among the 18 patients treated surgically, the duration of symptoms varied from two to twelve months in 13 and from one to six years in the others. The clinical diagnosis of testicular tumor was correct for 18 patients, while for 12 an incorrect diagnosis, such as hydrocele or tuberculosis, was made.

In 12,000 consecutive necropsies from 1929 to 1939 only 15 malignant tumors of the testicle were observed—12 of them dysgerminoma, 2 chorioepithelioma and 1 malignant teratoma. The 12 patients with dysgerminoma had metastases, 10 extensive, especially in the lymph nodes, lungs and liver, and 2 had hematogenous dissemination into the lungs, spleen, brain and bones. Thrombosis of the inferior vena cava and iliac veins occurred in 5. One patient was noted in whom each testicle seemed to be the site of a primary tumor, the second tumor developing twelve years after the removal of the first. Another patient showed metastasis to the opposite testicle.

The term "dysgerminoma" may be used to designate the testicular or ovarian tumors which heretofore have been termed "seminoma," "spermatocytoma" or "embryonal cell carcinoma." The origin of a dysgerminoma is controversial. Ewing and Hinman consider the tumor to be teratoid, although only one type of cell may exist. The explanation offered is that by rapid growth the type cell supplants and suppresses the other elements. Chevassu and Bell used the terms "seminoma" and "spermatocytoma" and stated their belief that the cells are derived from the lining of the seminiferous tubules. Robert Meyer believed that the cells are not derived from the tubules but that they invade the tubules. Other testicular tumors described as malignant teratoma usually contain various structures of mesoblastic, hypoblastic and epiblastic origin.

DISCUSSION

WALTER SCHILLER: If the term "dysgerminoma" is accepted it should be used as Robert Meyer suggested. He found the same kind of tumors in ovaries, unassociated with masculinization. He concluded that the growths arise from intersexual elements and produce no hormone. The prefix portion of the term implies origin in both sexes. Many of these growths appear with teratomas. In males the maximum incidence is between the ages of 30 and 40; in females between the ages of 10 and 15. The tumors in males are malignant; those in females, benign. In structure they are alike.

A. B. RAGINS: Were determinations of gonadotropic substance made?

J. D. KIRSHBAUM: Tests for such substance with 3 patients were negative.

CAUSAL SIGNIFICANCE TO TRAUMATIC OSSIFICATION OF THE FIBROCARTILAGE IN TENDON INSERTIONS. EDWIN F. HIRSCH and RUSSELL H. MORGAN.

The early stages of the lesion of traumatic ossification show large amounts of fibrocartilage or hyaline cartilage continuous with bone in varying degrees of differentiation. Some portions seem to be ossifying cartilage; others have lamellar trabeculae containing residues of cartilage. In the late stages the lesion has a high content of lamellar bone and only small traces of cartilage. These conditions imply that bone has its origin in a cartilage matrix, i. e., enchondral bone formation.

Fibrocartilage is a normal constituent of the insertions of many tendons in which traumatic ossification occurs. A reactive or reparative growth of these tissues initiated by trauma provides a simple explanation for the lesion of traumatic ossification.

The complete report will be published in the *Archives of Surgery*.

NEW ENGLAND PATHOLOGICAL SOCIETY

CHARLES BRANCH, *President*

Regular Meeting, April 20, 1939

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A MICROSCOPIC STUDY OF THE SPHENO-OCCIPITAL SYNCHONDROSIS IN MONGOLISM, WITH A COMPARISON OF THIS GROWTH DISORDER WITH CRETINISM AND CHONDRODYSPLASIA. CLEMENS E. BENDA, Wrentham, Mass.

Previous studies in mongolism by means of roentgenograms and anatomic measurements revealed a growth disorder which involves the proliferative growth of cartilaginous and membranous bones. The purpose of the present investigation was to determine the nature of this disorder of growth by microscopic study. The spheno-occipital synchondrosis of 6 mongoloid patients, aged between 8 months and 30 years, revealed a lack of preparatory column formation of the cartilage and an absence of growth sequences. The bone ridges were heavily ossified and the bony borders perforated by primary marrow cavities.

The sphenoethmoid synchondrosis was found to be open and without ossification in 3 cases. Two vertebrae of mongoloid children, 2 days and 6 months of age, respectively, were examined. That of the first showed an almost normal picture, while that of the 6 month old mongoloid child disclosed complete arrest of growth and formation of heavy transverse bone ridges.

A comparison of the bone changes observed in chondrodysplasia, cretinism and mongolism reveals differences in these three conditions. In chondrodysplasia there is a primary change in the cartilage with early absorption and ossification, whereas the membranous bones and sites of periosteal ossification are not involved. In cretinism the transformation of cartilage into bone is affected, but the preparation of the cartilage is normal. In mongolism there is a deficiency of growth in all epiphysial lines. The cartilage fails to grow normally or to be absorbed. This picture, together with lack of sinus formation in the bones of the skull, lack of diploe and absence of growth at the bone ends, is suggestive of congenital pituitary deficiency. Evidence for such a conclusion has been accumulated by a study of 14 pituitaries of mongoloids, reported previously.

TRAUMATIC EPITHELIAL CYSTS. THOMAS G. COGSWELL (by invitation) and RAYMOND H. GOODALE, Worcester, Mass.

Traumatic epithelial cysts, also called epidermoid or implantation cysts, occur more frequently than is usually recognized. Comparatively little has been written about them, especially in American literature, and they are barely mentioned in the textbooks of this country. The traumatic origin has apparently been accepted universally, but the epithelial source is still subject to controversy. Several theories have been advanced, but that which sees the development of such cysts from sudoriferous glands and their ducts seems to be the most satisfactory.

The cysts appear as small round or oval swellings, which are usually nontender and somewhat freely movable. The common sites are the palms of the hands, the flexor surfaces of fingers and thumbs, the soles and exposed areas of the face. The greater incidence is in males. Microscopically, the cyst wall strongly resembles the epidermis, and the contents are cellular débris and dense keratin. Traumatic epithelial cysts are most easily confused with sebaceous cysts. It should be noted, however, that they appear on areas devoid of sebaceous glands or hair follicles and also that they exhibit differences observable in microscopic examination.

These cysts rarely become malignant and seldom become infected. An epithelial cyst in the pulp of a thumb or a finger may erode into the phalanx. The treatment is excision. There is a tendency to recur if removal is not completely accomplished.

DISCUSSION

B. E. CLARK, Providence, R. I.: I fear all are prone to pass over commonplace and benign lesions too lightly, with a feeling that there is nothing more to be learned about them. Probably many are accustomed to group all these epithelial-lined cysts together as epidermal cysts or something similarly designated. One may have in mind the possibility that some are of traumatic origin, that others result from obstruction to glands and that others come from embryonal rests, but one makes little or no attempt to find morphologic differences that may indicate the origin. Then some one with a little more curiosity makes a careful study, such as Dr. Cogswell has just presented, and teaches one that there are morphologic differences which others have not noted or have failed to interpret.

F. P. MCCARTHY, Boston: At a large dermatologic clinic (50,000 visits and 8,000 new cases) my associates and I see only one or two of these epidermal cysts each year. I assume that most of the patients with such lesions go to the surgeon rather than to the dermatologist. Many are assumed to be sebaceous cysts and are treated accordingly, without histologic examination. Cysts of the type described do not tend to become infected as often as the sebaceous cysts. They are smaller, deeper, firmer, and often show a hyperkeratotic reaction of the skin covering the lesion.

LEO ALEXANDER, Boston: My colleagues and I have seen occasional instances of epidermoid tumor located intracranially. In the case of a woman, a report of which has since been published by Drs. Munro and Wagner, the tumor was found extradurally in the right frontal region. This woman gave a history of having incurred a skull fracture in early childhood, involving that region, and it is reasonable to assume that epidermal material may have been implanted in the epidural tissue at that time. In other cases the traumatic orign was less obvious — for instance, in those cases in which such a tumor was seen in the cerebellum.

Recently in an apparently similar case in which Dr. Munro operated two discrete, partly calcified nodules were found within the pia at the convexity of the brain posterior to the central region. However, in this case the differential diagnosis from small meningiomas with partial fatty degeneration has not yet been ascertained. I should like to ask Dr. Cogswell whether he has observed calcification in any tumor in his cases.

B. E. CLARK, Providence, R. I.: Dr. Cogswell mentions the foreign body reaction which occurs about some of these cysts. This is a common observation, and I have interpreted it as being due to a break in the epithelial lining with escape of the sebaceous content into the tissues, this material then stimulating the foreign body reaction. I wish to ask whether Dr. Cogswell feels that this is characteristic of cysts which are traumatic in origin. Or may it not occur with so-called wens or cysts of other origin?

J. E. PORTER, Fall River, Mass.: What is the interval from the time of the trauma until the cyst is recognized?

ROBERT FIENBERG, Westfield, Mass.: At one time I was interested in the contents of epidermal cysts and extracted the material with fat solvents. I found it to be predominantly keratin. This finding probably explains the giant cell reaction in the tissues about the cyst, since the keratin might penetrate the corium through a break in the epithelial lining. Certainly, when in heavily irradiated keratinized epidermoid carcinomas the cancer cells are destroyed, leaving necrotic masses of keratin, foreign body giant cell reaction is often noted about the keratin.

Assuming that these epidermal cysts arise from sweat glands, it is interesting that Dr. Cogswell was able to differentiate the epithelium found in the epidermal cysts from that found in the sebaceous cysts. Since both arise from cutaneous appendages, the assumption would be that the metaplasia which occurs in these cysts would give rise to the same type of squamous epithelium in each kind of cyst. Certainly, in most cases of squamous metaplasia one does not usually distinguish types of squamous epithelium.

RAYMOND GOODALE, Worcester, Mass.: We have seen no malignant tumors in our series. These cysts are prone to recur. The recurrences may be due to a failure of the surgeon to remove tiny diverticula of the cysts. Frequently these cysts are more difficult to excise than sebaceous cysts. Complete excision is essential for a cure.

THOMAS G. COGSWELL, Worcester, Mass.: Unfortunately we have seldom had complete clinical information. However, in a few cases we have observed hyperkeratosis of the overlying skin. No intracranial lesions have been seen in our series of cases. Reports of epidermoid cyst of the brain were found in the literature. The so-called cholesteatoma involving the middle ear region is regarded as a similar lesion. We have confined ourselves to a study of the cysts of the skin. It is logical to believe that giant cells may be present as a reaction to the dense masses of keratin if there is a break in the wall which would allow keratin and other material to be passed out into the surrounding tissue. It is interesting to note that no such phenomenon was found in any of the sebaceous cysts.

Replying to the questions concerning trauma, it should be pointed out that the injury may be so slight as to pass unnoticed. It may be said that a deep penetrating trauma is definitely not necessary for the development of such a cyst. The cause may be constant irritation or repeated trauma, and no time element can be determined. In certain instances there may be a single traumatic injury which is well remembered by the patient. Two or three months are usually required for the development of the cyst, but many months or perhaps years may pass before clinical symptoms are manifested. Certain factors, especially the site of the lesion, will have a bearing on the time element.

LESIONS OF THE NERVOUS SYSTEM IN HYPERINSULINISM. H. M. ZIMMERMAN, New Haven, Conn.

The experimental animal employed in this study was the cat. From 10 to 20 units of insulin per kilogram of body weight was injected, and by frequent analysis of the blood for sugar it was determined that the period of hypoglycemia extended for about ten to fifteen hours. About 30 per cent of these animals recovered completely, and about 40 per cent did not survive the procedure. The remaining

30 per cent survived but showed a condition approximating decerebrate rigidity. These animals were semiconscious, insensitive to painful stimuli, ataxic and spastic. Their blood sugar levels at this time were within normal limits.

Chemical analysis of the brains by Dr. Herman Yannet disclosed a marked loss of cell water and a consequent increase in extracellular water, but the total water of the brain was not changed. There was an even greater loss of cellular potassium, with a shift of sodium into the cell. These changes were interpreted as indicating a loss of selective permeability of the cellular membrane, probably due to cellular disintegration.

A close correlation was found between the severity of these chemical changes and the anatomic lesions in the brain. The latter consisted of widespread cortical necrobiosis affecting the ganglion cells, which at first showed liquefaction and pericellular encrustation and later disappeared. The same type of change was found in the cornu ammonis formations, the basal ganglions and the cerebellum. Under certain conditions, especially when the duration of the hypoglycemia was short, these changes were apparently reversible, and the animal recovered both functionally and anatomically. Changes which are identical with these have been observed in both rabbits and men under conditons of hyperinsulinism with resulting hypoglycemia.

The genesis of these lesions is probably explained on the basis of a lack of dextrose in the brain resulting in "cellular starvation", since the brain is dependent for its nutrition in great part on the dextrose brought to it by the blood. Cerebral anoxemia produced by vascular occlusion produces focal lesions rather than the diffuse involvement seen in hypoglycemia. The anoxemia produced by such convulsants as thujone, camphor and metrazol, even when severe, never leads to such widespread destruction of ganglion cells, even though the type of cell injury is the same.

DISCUSSION

QUESTION: Were hemorrhages found in the brains of these animals?

VALY MENKIN, Boston: In regard to the mechanism of cytolysis, have you had the opportunity to study the local changes in p_H? I have had no experience with the tissues which you are studying; but at least in exudates polymorphonuclear leukocytes are definitely injured by a fall in p_H. Incidentally, my coworkers and I also have been able to show that accompanying an inflammatory reaction there is an increase in the potassium of the exudate as compared with that of the serum. This is probably associated with injury to the cells. In our studies the local acidosis was referable to disturbances in the intermediary carbohydrate metabolism. This favors development of true local acidosis from lactic acid at the site of the injury. I wonder whether there is any possibility that a similar picture might present itself in Dr. Zimmerman's experiments on hypoglycemia. I should therefore be interested to know whether he has had the opportunity to study glycolytic changes at the site of ganglion cell degeneration.

LEO ALEXANDER, Boston: Dr. Zimmerman has been the first to demonstrate clearly that changes similar to those which one observes following occlusion of vessels and which one therefore tends to accept as due simply to anoxia may be brought about by lack of a substance other than oxygen in the circulation, namely, dextrose. Although the general distribution of the change is not similar to that of the change brought on by simple anoxia, the cytologic change is rather similar to the so-called ischemic change of Spielmeyer; particularly, the analogy of the change in Ammon's horn is most striking. Dr. Zimmerman's paper demonstrates most ingeniously that there are at least two components in the so-called ischemic change, that of anoxia and that of lack of dextrose.

We have had the opportunity in our laboratory to study a case of death following insulin shock in man. Death occurred in the acute stage of shock. An enormous degree of cerebral vasodilatation was observed in both upper parietal regions symmetrically. I should like to ask Dr. Zimmerman whether in his cases the vascular system showed gross morphologic abnormalities.

The first one to suggest that the apparent improvement in schizophrenia following a series of insulin shocks may be due to destruction of cerebral tissue, which allows adjustment on a lower integrative level, was Dr. Stanley Cobb, of Boston. I do not think that he intended necessarily to regard this fact as a strict contraindication to the treatment, but rather as a fact which might explain the mechanism involved in the apparent improvement. Dr. Zimmerman's paper and clinical experience have since confirmed Dr. Cobb's point of view. The question of whether the therapeutic use of insulin shock which destroys part of the brain is promoting the social adjustment of the schizophrenic patient will have to be decided from the study of a great amount of clinical material.

T. Dennie Pratt, Boston (by invitation): Recently, while working under the direction of Dr. Oliver Cope at the Massachusetts General Hospital, I carried out an experiment with 2 adult male dogs in which the period of hypoglycemia induced by insulin was prolonged for forty-eight hours; except for one or two short intervals the level of the blood sugar was kept well below 40 mg. per hundred cubic centimeters, with most of the readings being around 30 mg.

Profound objective neurologic changes appeared. Extreme prostration, together with spastic paralysis of the extremities, and complete failure to react to varying types of external stimuli were observed on the second day of the experiment. This stage was followed by a gradual return toward the normal, during which the dogs exhibited fairly typical examples of the rage phenomenon described by Cannon as being characteristic of the decorticate animal. In the succeeding two to three weeks the dogs slowly but definitely returned to their original normal state. No histologic studies have as yet been made.

In view of these objective findings I should like to ask Dr. Zimmerman if he thinks it likely that these dogs could have had at one time the histologic changes which occurred in his cats, since the dogs now appear to have recovered completely. If so, one might conclude that either (1) the histologic changes are temporary or (2) the brain of the adult dog has a certain supplementary reserve by means of which the unaffected cells are enabled to take over the functions of the cells permanently damaged by the extreme hypoglycemia.

H. M. Zimmerman, New Haven, Conn.: Only two of the animals had hemorrhages, insignificant ones, and none of them showed any striking vasodilatation. Determinations of p_H on some of the more severely involved animals showed changes to the acid side. These changes, however, may have been the result of the extensive cellular destruction rather than the factor responsible for them. Animals which were hypoglycemic for less than four hours usually showed normal responses almost immediately thereafter. Those that were hypoglycemic for much more than four hours remained out of touch with their environment until put to death.

Replying to the question concerning areas of involvement — there appears to be no predilection for the parietal lobe. If anything, it is the occipital lobe that suffers the most.

One question pertained to clinical evidence indicative of cerebral injury in patients treated with insulin shock. Recently, I have been informed by Dr. Fritz Kant, who has had experience with a large number of such patients, that following repeated shocks even those patients who respond favorably show evidence of this injury. Psychologic tests indicate that they are brought to a lower intellectual plane by this form of therapy.

Arteriosclerosis. M. C. Winternitz, New Haven, Conn.

The extensive vascular supply to the wall of artery and vein necessitates consideration of the role this may play in the disease of the vessel wall. Hemorrhages are frequently encountered in all of the coats, particularly in the intima. The fate of this hemorrhage can be traced through the breakdown products of the erythrocytes. These stain for iron and for fat in various stages of their

disintegration. This is so for some cells still identifiable in the atheroma and in vessels at its periphery both when they are free and when they are within the bodies of large macrophages. All of the iron within the macrophages probably is derived from the red blood cells. The fat may well arise from other sources. That much of the fat within the macrophages may be derived from red blood cells has been demonstrated by the injection of various irritants into the peritoneal cavities of rabbits. When red blood cells occur in the exudate, the macrophages are laden with fat.

Intimate association between artery and neighboring vein has been established by injection and clearing methods. When the vein wall or the adventitia of the vein is inoculated with various micro-organisms, lesions of both the vein and the artery follow. These are dependent largely on the virulence of the organisms. In the vein, acute phlebitis and also thrombosis may result, and later, if the animal survives, either organization of the thrombus or a fibrous intimal plaque follows. The arterial lesion is associated with exudation, more marked in the adventitia and in the intima than in the media. Intimal proliferation follows rapidly. Thrombosis of the artery may occur, or if the process does not extend to the lumen of the artery, it may result in progressive thickening of the intima. Vascular lesions have been found to occur in another and quite different condition. Hypertension and azotemia, produced by the Goldblatt method, are associated with lesions of the media of the larger arteries and veins. Edema of the media and extravasation of blood in this coat of the vessel, extending into the intima but without loss of continuity of the intimal surface, occurs frequently in these larger vessels. This is the analogue of the arteriolar lesions that occur under these circumstances, as described by Goldblatt and his associates.

DISCUSSION

H. E. MacMahon, Boston: I should like to ask Dr. Winternitz what procedure he follows in obtaining these very striking pictures showing vascularization with hemorrhage throughout the wall of the aorta; also, what part of the aorta most frequently shows these lesions, and at what age they most commonly occur.

In a study of the vascular lesions of lungs in cases of long-standing pulmonary tuberculosis one may find within the vascular walls every change that has been shown here. In a comparatively limited study of the aorta in younger patients showing marked hypertension I have been unable to find such striking pictures as have been shown by Dr. Winternitz. This, I feel, is largely due to the technic, for the clearing process was not used in the selection of sites for study.

In a recent paper Neumann and co-workers (*Virchows Arch. f. path. Anat.* **303**:1, 1938) pointed out that the first portion of the aorta, extending up to the arch, is supplied by branches from the coronary arteries. I should like to ask Dr. Winternitz if he has had an opportunity to study this particular vascular tree.

Clemens E. Benda, Wrentham, Mass.: I should like to ask Dr. Winternitz if any type of infectious process produces the lesions in blood vessels which he has shown to us; also how widespread they are.

B. E. Clarke, Providence, R. I.: I should like to ask whether or not vascular lesions of this type may serve in explaining idiopathic cystic medial necrosis.

M. C. Winternitz, New Haven, Conn.: Clearing of the vessel wall is of great importance in demonstrating hemorrhage. Extensive sectioning of the vessel wall with a sharp instrument is also of great aid. It should be emphasized that in such manipulations damage to the intima by such procedures as sponging and washing should be avoided. The iron stain as well as stains for fat are of value in demonstrating the participation of red blood cells in the process of disease of the vessel wall. A possible relationship between these medial hemorrhages and idiopathic medial necrosis may be suggested.

Book Reviews

Problems of Ageing: Biological and Medical Aspects. Edited by Edmund Vincent Cowdry, A.B., Ph.D., Professor of Cytology, Department of Anatomy, Washington University. Cloth. Pp. 749, with 121 illustrations. Price $10. Baltimore: Williams & Wilkins Company, 1939.

The book is a product of the interest of the Josiah Macy Jr. Foundation in chronic degenerative conditions and impairments with special reference to aging. As stated in the editor's preface, the book is a logical sequence of the survey of the problem of arteriosclerosis issued under the same auspices in 1933, in which is summarized the knowledge of degenerative changes and aging of blood vessels (Cowdry, E. V., editor: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933). In his foreword, Lawrence K. Falk, of the Foundation, describes the general scope and purpose of the book as conceived and carried out by the editor, namely, to advance the "larger conception of health-care to which the Foundation" is dedicated. The book deals not only with the involutions of uncomplicated aging—"the gradual running down of the hereditary 'time-clock' "—but inevitably also with changes in the aged due to degenerations and impairments other than those inherent in physiologic aging. This distinction and the interrelations of the two sets of processes naturally give rise to much discussion. In his introduction, John Dewey emphasizes the relations between aging and the social life. He says: "Science and philosophy meet on common ground in their joint interest in discovering the processes of normal growth and in the institution of conditions which will favor and support ever continued growth." There are 25 chapters in which phases and problems of aging are discussed by well known investigators in various fields. A list of the chapter headings and authors will show best the ground work covered:

Ageing of Plants—William Crocker
Senescence and Death in Protozoa and Invertebrates—H. S. Jennings
Ageing of Insects—L. O. Howard
Ageing of Vertebrates—T. Wingate Todd
Human Cultural Levels—Clark Wissler
Longevity in Retrospect and Prospect—Louis I. Dublin
Cardiovascular System and Blood—A. E. Cohn and Karl Landsteiner
Lymphatic Tissue—E. B. Krumbhaar
Digestive System—A. C. Ivy
Urinary System—Jean R. Oliver
Skeleton, Locomotor System and Teeth—T. Wingate Todd
Ageing of the Skin—F. D. Weidman
The Thyroids, Pituitary, Adrenals, Thymus and Islands of Langerhans—A. J. Carlson
Female Reproductive System—Edgar Allen
Male Reproductive System—Earl T. Engle
Changes in Personality and Psychosexual Phenomena with Age—G. V. Hamilton
Ageing of the Nervous System—Macdonald Critchley
The Eye—Jonas S. Friedenwald
The Ear—Stacy R. Guild
Psychological Changes—Walter R. Miles
Chemical Aspects of Ageing—C. M. McCay
Ageing of Homeostatic Mechanisms—Walter B. Cannon
Ageing of Tissue Fluids—E. V. Cowdry
Ageing Processes Considered in Relation to Tissue Susceptibility and Resistance—Wm. DeB. MacNider
Ageing from the Point of View of the Clinician—Lewellys F. Barker.

Each chapter concludes with a summary and a bibliography. For the editor and the contributors there can be nothing but praise. The book gives an excellent and unique recapitulation of the knowledge and understanding of the processes and manifestations of aging. It clarifies the present state, trends, and need of investigation in the field of aging.

William B. Wherry, Bacteriologist. Martin Fischer. Pp. 293. Springfield, Ill., and Baltimore, Md., Charles C. Thomas, Publisher, 1938.

This book is written for those who knew and loved the rare spirit that in the flesh was called William B. Wherry; it is not primarily for those whose chief interest would be to learn of the bacteriologist and scientist. The sources for material have been a vast accumulation of letters, 82 articles written in whole or in part by Wherry and an intimate friendship between Wherry and the author that began in medical school and lasted some thirty-five years, until the former's death in 1936. Skilfully drawing now on one source and now on another, Fischer tells of Wherry's boyhood interest in nature, his struggles with poverty, his strong home attachments and the gradual growth of more liberal views as to life than those held by his missionary parents. The student days at Rush Medical College are described, and the early contact with Hektoen, whose wise advice was for years an encouraging and much appreciated stimulus to productive research. One follows Wherry to Manila, California, Montana and many other places until finally, after wandering Odysseus-like in many lands, he reaches what was to be his final home in Cincinnati, where, as one of a congenial and understanding group, he continued his investigations and became an inspiring teacher, a leader in medical circles, an honored citizen and a beloved colleague and friend.

The author's task has been a labor of love. No incident is too trivial to record if it shows the dutiful son, the loving husband or the loyal friend. No excerpt from his writings is insignificant if it adds to the credit account of Wherry's investigations and discoveries in the field of bacteriology. Leprosy, plague, tularemia, amebiasis; improved culture mediums and methods of staining; more accurate technic for animal experimentation; original ideas as to the causation of disease; problems of immunity; the growth of a broad philosophic outlook on the nature of man and his diseases—all this is told with the charm of the easy style for which Martin Fischer has become well known. Occasionally there is pungent irony to an epigram. But the "Fischerisms" are not viciously barbed. The author is evidently trying to exercise restraint lest he be viewed as a too ardent advocate or an undiscriminating hero worshiper. Some critics may, in fact, think he is at times extravagant. He tacitly endorses the statement of another that when Wherry died "the last and the greatest of the bacteriologists of our period" had gone. Such critics might—naming only one—cite Theobald Smith as in all respects a greater bacteriologist. Theobald Smith, by the way, was one with whom Wherry came in contact and whose helpful influence he freely acknowledged. The number of other friends was great. Hektoen has already been mentioned. Others were Fischer, Ricketts, E. O. Jordon, Gideon Wells, Rosenow, Musgrave, Strong, McDill, as well as many unnamed college and hospital colleagues and students.

While, as the title of the biography declares, Fischer is considering Wherry chiefly as a bacteriologist, it is clear that he regards him—and readers must admit that he makes out his case—as worthy of remembrance not alone because he achieved renown in his chosen field of science but as well because he stimulated to sane thinking, advocated a broad philosophic view of life and by example and precept taught the value of mutually helpful friendship. It is a pleasure to add that the volume has been brought out in a form that is a credit to the publishers as well as to the author.

Books Received

THE BIOLOGY OF BACTERIA: AN INTRODUCTION TO GENERAL MICROBIOLOGY. Arthur T. Henrici, M.D., Professor of Bacteriology, University of Minnesota. Second edition. Cloth. Pp. 494, with 112 illustrations. Price $3.60. Boston: D. C. Heath & Co., 1939.

LE SÉRUM NORMAL. PROPRIÉTÉS PHYSIOLOGIQUES. D. Brocq-Rousseu and Gaston Roussel. Volume 2. Pp. 630. Price 140 francs. Paris: Masson & Cie, 1939.

LABORATORY MANUAL OF THE MASSACHUSETTS GENERAL HOSPITAL. Francis T. Hunter, M.D., Associate Physician and Clinical Pathologist, Massachusetts General Hospital, Boston. Third edition, revised. Pp. 119. Price $1.75. Philadelphia: Lea & Febiger, 1939.

APPENDICITIS: A STATISTICAL STUDY. Matthew Young and W. T. Russell. Medical Research Council, Special Report Series, no. 233. Paper. Pp. 64. Price 30c. His Majesty's Stationery Office, 1939.

ENDOCRINOLOGY IN MODERN PRACTICE. William Wolf, M.D., M.S., Ph.D., Endocrinologist to the French Hospital and Attending Endocrinologist to the Misericordia Hospital, New York; Consulting Endocrinologist, New York University Dental School. Second edition, completely revised. Cloth. Pp. 1077, with 176 illustrations. Price $10. Philadelphia: W. B. Saunders Company, 1939.

LA RECHERCHE DE LA PATERNITÉ PAR LES GROUPES SANGUINS. ÉTUDE TECHNIQUE ET JURIDIQUE. Louis Christiaens. Paper. Pp. 108. Price 26 francs. Paris: Masson & Cie, 1939.

LA TUBERCULOSE DU COBAYE. Jean Paraf Médecin des Hopitaux de Paris. Paper. Pp. 132. Price 30 francs. Paris: Masson & Cie, 1939.